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OF

THE HARVARD MEDICAL SCHOOL

BOSTON
MASSACHUSETTS
1907

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THE FOURTH REPORT

OF

THE CAROLINE BREWER CROFT FUND

NOVEMBER 1, 1907.

DEAR SIR:

The Fourth Report of the Cancer Commission of the Caroline Brewer Croft Fund is herewith submitted for your consideration. Should you desire to receive the future reports of this Commission as they may be issued, will you kindly acknowledge the receipt of this report, and send your full address to the Secretary at your earliest convenience.

For those who care to collect and bind in one volume Reports Numbers 1, 2, 3, and 4 of this Commission, a title-page and table of contents covering the four reports are published at the end of Report No. 4.

The Commission regrets that the editions of Report No. 1, published in October, 1900, and of Report No. 2, published in February, 1902, are exhausted. Any copies of these reports will be gratefully received and acknowledged by the Secretary.

Very truly yours,

J. COLLINS WARREN, *Chairman.*

ROBERT B. GREENOUGH, *Secretary,*
Harvard Medical School, Boston.

◆

THE HARVARD MEDICAL SCHOOL
BOSTON, MASSACHUSETTS

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THE FOURTH REPORT

OF

THE CAROLINE BREWER CROFT FUND CANCER COMMISSION

OF

Univ.

THE HARVARD MEDICAL SCHOOL

J. COLLINS WARREN, CHAIRMAN

HENRY P. WALCOTT ARTHUR T. CABOT
For the Corporation of Harvard College

HENRY K. OLIVER J. COLLINS WARREN
For the Croft Fund

ROBERT B. GREENOUGH, SECRETARY

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DIRECTOR OF RESEARCH FOR THE
FOURTH REPORT

ERNEST E. TYZZER.

CONTENTS

PAGE

INTRODUCTION.
J. Collins Warren 5

THE INOCULABLE TUMORS IN MICE.
Ernest E. Tyzzer 9

A SERIES OF TWENTY SPONTANEOUS TUMORS IN MICE, WITH THE ACCOMPANYING PATHOLOGICAL CHANGES AND THE RESULTS OF THE INOCULATION OF CERTAIN OF THESE TUMORS INTO NORMAL MICE.
Ernest E. Tyzzer 27

A STUDY OF HEREDITY IN RELATION TO THE DEVELOPMENT OF TUMORS IN MICE.
Ernest E. Tyzzer 71

THE FOURTH REPORT

OF

THE CAROLINE BREWER CROFT FUND CANCER COMMISSION

OF

THE HARVARD MEDICAL SCHOOL

INTRODUCTION.

J. Collins Warren.

In presenting the Fourth Report of The Caroline Brewer Croft Fund Cancer Commission, the committee wishes to call attention to the work which has been covered in previous reports, and to the different phases of the question of the causation of cancer which have been attacked.

The First Report was issued in 1900. At that time there were many questions of cancer etiology which demanded discussion and solution. The question of the parasitic origin of cancer obtained then a much more general credence than it does now. There were reports from various sources of microörganisms of one kind or another, which it was claimed played a prominent part in the cancer process. Prominent among these we may refer to the so-called "Plimmer's Bodies," the blastomycetic group of organisms, various bacteria, and the so-called sporozoa of certain animals. A proper consideration of these topics required a study of the field of work by a number of individuals, and the First Report accordingly contained papers upon several of these subjects by Drs. E. H. Nichols, R. B. Greenough, and E. E. Tyzzer. A series of experiments were also made by Dr. Oscar Richardson to determine whether any of the bodies found in the cells of carcinoma could be made to grow in nutrient media.

These investigations were continued in the Second Report, published in 1902. Studies were made of the coccidium infection in the rabbit, and of the histological appearances in the disease known as molluscum contagiosum. Investigations of the relation of blastomycetes to cancer were pursued still further, and the nature of the cell inclusions in cancer, which at that time caused much discussion amongst those engaged in this work, was studied and analyzed.

The Third Report appeared in 1905, and contained further contributions to the study of cell inclusions, and an extensive series of experiments upon the implantation of tissue in living animals. Professor F. B. Mallory made in this report a contribution to the classification of tumors by means of a study of cells and intercellular fibrils by special staining methods. The Third Report also entered somewhat into the study of the clinical side of the cancer question with an investigation of the extent to which the action of the Röntgen ray was of value from a therapeutic point of view.

The Commission feels that the work done thus far has been of considerable value, and that the conclusions deduced from the investigations made have withstood, in a most satisfactory way, the further test of time and discussion. It may be said that this work has aided in the settlement of many of the vexed questions which arose at the outset of the scientific investigation of this disease, and that by removing from further consideration some of the unsubstantiated theories of the parasitic origin of cancer, has contributed toward the ultimate elucidation of the great problem of the origin of this disease.

In the earlier reports the investigations of the Commission were confined almost entirely to the study of cancer from the pathological and histological point of view, and animal experimentation was employed only secondarily and with only negative results. The discovery by Professor Jensen of the inoculable carcinomata of mice, and the stimulus given thereby to experimental research upon cancer problems, opened a new field of activity which was promptly taken up by many commissions and institutions devoted to cancer research. It is to the results of this investigation that the Fourth Report is devoted.

By means of these inoculable mouse tumors the investigator is able to study the natural history of the disease, and to observe the cancer cell itself under various conditions as an actively growing parasitic organism. Not only can the relations of the cancer cells to the other tissues of the body and the different stages in the life history of the cell itself be studied, but the possible causes —

parasitic, chemical, or whatever they may be — which from time to time play a part in the phenomena of cell growth, may be approached. During the course of these experimental investigations, which have now become most extensive, many incidental data of extreme interest have been obtained bearing upon such topics as the natural or acquired resistance of the body tissues to infection with cancer cells, natural and acquired immunity, and even to the possible curative action of the blood or serum of recovered animals.

The Commission recognized the importance of approaching the subject of cancer research from an etiological point of view in the early stages of their investigations, but does not wish to be regarded as considering lightly the importance of the study of the disease from a clinical standpoint. To carry on clinical investigations on a scale which would bring out new and valuable data would require resources far beyond what most commissions have at their disposal. It is along these lines of investigation that large endowments would enable us to place the clinical and the laboratory work in close juxtaposition in such a way as to produce results which it is not possible to obtain either at the hospitals, or with the material of private practice which practitioners of the present day have at their disposal. Special wards for the study of cancer involve a large expenditure of money. The disease should be studied not only in its incurable stages, but at its very inception, when it is most susceptible to the action of remedial measures. Such institutions should be planned also to train men especially for cancer work. The Commission feels that the investigation of cancer has now reached a point where it is possible to take a new departure in this direction on a far more extended scale than has ever been attempted, and on lines even broader than those which have been laid down for the study and control of tuberculosis. Such institutions would not only be productive of valuable scientific data, but would train men who would be of the greatest value to the public health of the community from a protective standpoint. A moderate portion of the vast sums of money which are being used in the crusade against tuberculosis would be of the greatest value at the present moment in the attack upon perhaps the next most dreaded disease of modern times.

The report which the Commission presents in its fourth publication is based upon a series of observations and experiments conducted by Dr. E. E. Tyzzer, especially with reference to the inoculable tumors of mice. Concentration upon this subject has been made possible by the elimination of many other problems which at first presented themselves as has been detailed in previous reports, and the work has been greatly stimulated by the researches carried on in other laboratories and under other commissions along this line. The Commission is glad to acknowledge, gratefully, the assistance which it has received from other laboratories devoted to cancer research. Especially would it express thanks to Professor C.O. Jensen of Copenhagen; Dr. E. L. Bashford of the Imperial Cancer Research Commission of London; Professor Ehrlich of Frankfurt; Professor Borrel of the Institute Pasteur of Paris, and to Drs. Park, Gaylord, and Clowes of Buffalo.

THE INOCULABLE TUMORS OF MICE.*

E. E. Tyzzer.

The possibility of the artificial propagation of tumors, in certain animals, by the inoculation of tumor tissue into normal individuals of the same species was first shown in the experiments of Hanau,[15] who employed a carcinoma of a rat in his inoculations. In a similar manner, Morau[21] subsequently succeeded in propagating by inoculation an adeno-carcinoma of a mouse, and Loeb[17] a sarcoma of a rat. Jensen[16] in carrying on a series of inoculations with a carcinoma of a mouse demonstrated, by a histological study of the tissue taken at intervals after inoculation, that the epithelium of the tumor appearing in the inoculated animal is derived from the growth of the injected tumor epithelium, and not from the proliferation of the tissues of the inoculated animal. Bashford[1] made a thorough histological study of the changes taking place in and around the tumor tissue after its inoculation into normal mice, and confirmed Jensen's conclusions. Other investigators working upon this subject have become convinced that the development of these tumors in the animals inoculated is dependent upon the transplantation of tumor cells.

Adeno-carcinomata of mice have also been propagated by Borrel,[4] Haaland,[14] Ehrlich,[7, 8, 9, 10] Michaelis,[20] Bashford,[1, 2, 3] Loeb,[18] and others. These adeno-carcinomata are subcutaneous in origin, and have usually occurred in female mice. Ehrlich and Apolant[7] have obtained inoculable sarcomata which originated in their series of carcinoma inoculations. Although the original tumor was carcinoma, certain of the tumors resulting from the inoculations were of mixed type and consisted of both epithelial and connective tissue elements. These mixed tumors on further inoculation gave rise, in certain instances, to pure sarcomata. Loeb[18] had obtained similar

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* Received for publication Aug. 29, 1907.

results with a tumor of a Japanese waltzing mouse. Ehrlich[9] has also propagated a chondroma of a mouse. There is as yet no unity of opinion concerning the nature of the so-called infectious lympho-sarcoma which occurs frequently in dogs.[*] Some of the investigators are convinced that the tumor nodules arise from the proliferation of the tissue of the inoculated animal; others believe that the nodules arise from the growth of tumor cells which are introduced into the body of the normal animal by the act of inoculation.

The demonstration of the inoculability of certain tumors in animals has opened a new field of research in that an experimental basis is furnished for the investigation of various problems relating to tumor growth. Much has been done with the end in view of producing in animals an artificial immunity to the inoculable tumors, and results of considerable theoretical interest have been obtained. It has been found that in certain mice the inoculated tumors grow for a time, but later disappear spontaneously. Such mice are immune to subsequent inoculations. Various methods have been employed in attempts to reduce the vitality of the tumor tissue before inoculation so as to produce an artificial immunity. Michaelis has treated the tumor with chloroform before injection, and Clowes[6] has employed potassium cyanide, mercuric chloride, and other chemicals. Ehrlich produces immunity to the inoculable tumors by inoculating mice with a carcinoma of the hemorrhagic type, which grows in not over .2 per cent of the mice inoculated. The animals treated in this way are in nearly all cases protected against the more virulent strains of tumors subsequently inoculated. Ehrlich explains these results on the hypothesis of his "side chain" theory, and believes that, in these instances, an antibody is produced. The immunity brought about by the injection of one tumor apparently protects against all others. Thus he found that carcinoma inoculations protect mice against subsequently inoculated sarcoma and vice versa. Likewise tumor material injected into a mouse which already possesses a tumor invariably fails to grow. By inoculating rats with mouse carcinoma, Ehrlich found that the tumor epithelium grew for a time, but later disappeared. Rats injected in this manner developed an active immunity, and tumor epithelium subsequently inoculated failed to grow. If the tumor tissue, during its period of active growth in an inoculated rat, was transplanted into another rat, it failed to develop further, but if transferred back to a mouse it developed into a tumor. From these facts he infers that the rat tissues lacked an "X" substance which stimulates the tumor cells to active growth. This substance is found in the tissues of the mouse, and enough of this substance is carried over with the inoculated tumor tissue to enable it to grow for a short time in the body of the rat. This is demonstrated by the failure of the tumor tissue to grow when transferred to a second rat. Thus the rat, before it develops an active immunity, possesses what Ehrlich terms an "atreptic" immunity (τρεφω, to nourish).

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[*] Reviews of the literature bearing on this subject are to be found in the recent articles of Ewing and Beebe,[11] Bashford,[1, 3] and Borrel.[5]

It will be of interest to determine whether an artificially produced immunity to inoculable tumors serves as protection against the subsequent development of primary tumors. In a series of spontaneous tumors described in another section of this report, seven of the sixteen mice had been previously inoculated with tumor tissue of some sort. Whether the primary tumors in these cases were present previous to or developed after the inoculation — a point which, on account of their location, could not be determined — it appears either that the injection of mice with tumor tissue does not prevent the development of primary growths, or that the presence of primary growths in mice do not prevent successful inoculation. In two of the mice in which primary tumors were found, the inoculated tumors grew to large size.

Recent observations of Flexner and Jobling[12] indicate that certain properties of the inoculable tumors of mice may not be common to all tumors. They have worked with a sarcoma, inoculable in rats. This tumor grows when inoculated into rats which already possess tumors from previous inoculation. They also find that the growth of this tumor is increased by the injection of tumor juice into the animal.

Regarding the inoculation of tumor material into individuals of another species, most investigators have obtained negative results. Nichols[22] in the third report of the Caroline Brewer Croft Cancer Commission gives a comprehensive review of this question. All attempts made by him to transplant human cancers to rabbits and guinea-pigs resulted negatively. Mayet,[19] however, claims to produce tumors in dogs by the inoculation of human tumors. Since results such as these are infrequent, they require further confirmation by other investigators. Tumors are not infrequently met with in uninoculated animals kept for long periods of time under observation. There also appears to be some discrepancy as to what is to be regarded a tumor. Sanfelice[28] claims to produce tumors in animals by the inoculation of blastomycetes and their soluble products. The plates accompanying Sanfelice's article present no evidence that he is dealing with a true tumor, and it seems probable that the nodules which he obtains are infectious granulomata.

Opportunity is afforded, with animals kept constantly under observation, for obtaining data concerning the infectiousness of tumors. Tumors have occurred with such frequency in certain lots of mice often derived from a single cage that, to many investigators, it has appeared as evidence of infection. Such occurrences have been reported by Michaelis,[20] Haaland,[14] and Gaylord and Clowes.[13] Bashford,[1, 3] however, finds no evidence of infection in the development of tumors. He furthermore calls attention to the fact that the failure of tumors to develop in mice placed in cages in which tumor mice have been kept is not due to the absence of a suitable soil on which the virus may grow, for the exposed mice may by inoculation be demonstrated susceptible to the inoculable tumors. The increase in the virulence of certain inoculable tumors after being incubated for a time at temperatures from 38.5° to 41° C. as claimed by

Clowes[6] would appear to favor the presence of a living organism. No one, however, has been able to separate the hypothetical virus from the tumor cells, and, if a virus is present, it is destroyed by agencies which destroy the tumor cells. The evidence thus far furnished in favor of cage infection appears too meager to be conclusive. There has been no attempt on the part of the supporters of this theory to make thorough control observations, and the factor of heredity has been practically ignored. If mice are kept under observation for sufficiently long periods of time, tumors frequently appear among them even under conditions unfavorable to infection.

The following data, which I have obtained while keeping the Jensen and the Ehrlich " Stamm 11 " tumors, are given in brief. No attempt has thus far been made to carry out inoculation experiments on such an extensive scale as has been done in other laboratories. The results, although meager are in the main confirmatory of those of other investigators. The results of the experimental inoculation of certain primary tumors, which have arisen spontaneously in mice kept under observation, are given in another section.

During the summer of 1905 mice inoculated with the Jensen strain of tumor were received from the Imperial Cancer Commission of Great Britain and from the Gratwick Laboratory for the Investigation of Cancer, Buffalo; a mouse inoculated with a Borrel strain of tumor from the Pasteur Institute; and mice with tumors designated " Stamm 11 " from Professor Ehrlich. Both the Jensen and the Ehrlich tumors have been successfully transplanted from mouse to mouse and are growing at the present time. The mouse with the Borrel tumor died soon after its arrival, and the inoculation of the nodule which it presented into other mice was without result.

The technic at first employed in the inoculation of these tumors was to crush the tumor material in a small mortar, add a small amount of .6 per cent solution of sodium chloride so as to make it fluid, and then inject subcutaneously by means of a hypodermic syringe. This method was afterwards given up, and instead the tumor material was, without any addition of fluid, drawn into a small trochar and deposited beneath the skin of a mouse to be inoculated. In instances in which the tumor to be inoculated was infected with bacteria, it was found that by freezing it several times with carbon dioxide or by keeping it frozen over night, the tumor was still capable of growth, while the bacteria were apparently rendered inert. That the tumor epithelium is relatively more resistant than other tissues to low temperatures is readily shown by a comparative histological study of the other organs of the mouse frozen together with the tumor. All the organs show marked changes, and the liver tissue is scarcely recognizable. The frozen tumor epithelium, on the other hand, shows but slight change and appears to be living.

I. Inoculations with the Jensen tumor.

The Jensen tumor has been carried, during this period of investigation, for ten generations or transplants and has invariably retained its original type. With the exception of a few cases, a histological study was made of all tumors produced by inoculation. Seventy-three tumors have resulted from the inoculations and of these eleven (sixteen per cent), after growing for a period, have diminished in size and have ultimately disappeared. Certain of these were taken for histological study when about to disappear.

The Jensen tumor is regarded by most observers as a carcinoma. Its epithelium is arranged in solid masses or lobules which present nothing suggestive of glandular structure. The epithelium in the interior of the lobules — that is, in those portions farthest from the source of nutrition — tends to undergo necrosis (Fig. 3). Tumors which have attained considerable size are usually largely necrotic. In these the necrotic portion most often occurs in the interior of the tumor nodule, where it appears as dry, cheesy material, while the living tumor tissue is found at the surface. In a few tumors, however, the living and necrotic tissues are intimately intermingled. The stroma of the tumor consists of a rather loose type of connective tissue. The tissue surrounding tumor nodules is often edematous and more marked inflammatory processes are not uncommon. These changes do not appear to represent a reaction to the growth of the tumor, but rather secondary processes attendant upon necrosis or bacterial infection of the tumor nodule.

Evidence of growth in tumor material may appear soon, or a long period after the inoculation. The mass deposited from the trochar is usually perceptible on palpation during the week following the inoculation. In cases where there is no inflammation about the inoculated mass it may from the first show a gradual increase in size. On the other hand, the injected mass may disappear within a fortnight or persist for a longer time and then disappear. In many such mice growth never takes place, but in others growth appears after a long period in which there is no perceptible mass or thickening at the site of the inoculation. The longest period observed between the inoculation and the first evidence of growth was sixty-four days. In this case a mass, developed on the back at the site of the inoculation, grew for a short time, and then disappeared. In several other cases the tumors first appeared five or six weeks after the inoculation. Of those tumors which appeared late, nearly all grew for a short time, then diminished in size and disappeared. There were two tumors, however, of which one appeared fifty-one days after inoculation, grew rapidly to large size, and produced metastases in the lungs; the other appeared sixty days after inoculation, and also attained great size. There were, also, several others which appeared four or five weeks after inoculation, and grew slowly, but eventually attained considerable size.

The rate of growth varies greatly. Tumors produced in a single litter of mice, all inoculated with the same tumor, show great variation, some of them growing rapidly while others may grow very slowly. Usually those tumors which appear early after the inoculation show the greatest rapidity of growth.

The factor of age has been considered in the growth of inoculable tumors. Tumors have been produced in both old and young mice by inoculation. Of three mice inoculated when twenty-one days old, one developed a tumor which first appeared sixty-four days later, that is, at the age of eighty-five days. The rapid development and quick maturity of mice make it a difficult matter to determine the susceptibility in early life, for the reason that these animals become mature before the tumor is given sufficient time in which to grow perceptibly. In the case above mentioned, it seems possible that some condition essential to the active growth of the inoculable tumor is lacking in the early life of the mouse. It is my experience that the inoculation of the tumor into vigorous young adult mice results in growth in a larger number of cases than in old mice.

Metastases were found in four of the seventy-three cases of Jensen tumors of this series. The occurrence of these metastases was as follows: A mouse (designated 321 C) which had been inoculated with the Jensen tumor was received from the Buffalo Laboratory. This mouse presented several subcutaneous nodules and microscopic metastases in the lung. Ten mice were inoculated with the tumor of this mouse (321 C) with the result that tumors developed in eight, of which three had metastases in the lung. The tumor in one of the cases with the metastases was inoculated into two susceptible mice and produced rapidly-growing tumors in both cases, with metastases in the lungs of one. Thus these four cases with metastases were derived directly from a single case with metastases. In each case in which metastasis occurred, the growth of the primary tumor at the site of inoculation was extremely rapid, attaining the size of a pigeon's egg within four weeks. The metastases were found when the four mice were killed, respectively, twenty-eight, forty-one, forty-six, and forty-nine days after the inoculation. The size attained by the metastases corresponds quite closely to the interval which had elapsed since the inoculation (Figs. 2, 4, and 5). Thus the metastases found in the case taken twenty-eight days after inoculation were very minute and consisted of groups of tumor cells occluding small blood vessels; in the cases taken forty-one and forty-six days after inoculation the metastases were apparent to the naked eye, although very minute; and in the case taken forty-nine days after inoculation the lungs were studded with nodules, the largest of which measured five millimeters in diameter.

In this series of Jensen-tumor transplantations, metastasis invariably resulted from the dissemination of the tumor cells by way of the vascular system. The tumor material was in each instance inoculated subcutaneously, and in most cases the lymph nodes adjacent to the tumor were examined histologically. In no case were they found to be invaded by the tumor. In the lung the constant relation of the metastatic nodule to the pulmonary arteries leaves no doubt as to the mode of conveyance. In certain instances emboli consisting of tumor epithelium were found within the blood vessels. The tumor tissue may grow for a time within the vessel forming a mold of its cavity. If the tumor material only partially fills the vessel, its surface becomes covered with endothelium. The tumor continues to increase in size, dilates the vessel to its utmost, and finally grows out through the wall of the vessel. In some cases the elastic laminæ of the wall of the vessel are separated by layers of tumor epithelium. Even in nodules of considerable size the vessels from which they grow are readily demonstrated. If sections are stained with an elastic tissue stain the walls of the vessel from which the tumor has grown will be found to be outlined by the elastic tissue (Fig. 6). After breaking through the wall of the blood vessel the tumor epithelium fills the perivascular lymphatic, but is not confined to any extent in this space, for, apparently on account of the slight resistance of the lung tissue, it grows by expansion in all directions. After the metastatic nodules attain a certain size they acquire a stroma and blood supply of their own. The central portion of the nodules commonly becomes necrotic, and the metastases are identical in character with the subcutaneous growth developing at the site of inoculation.

Certain breeds of mice were found insusceptible to the Jensen tumor. The occurrence of mice insusceptible to the inoculable tumors has been noted by various investigators. Some, however, have found that this natural immunity may be offset, after a time, by the adaptation of the tumor to the special breed of mice. Thus, where but few tumors resulted from the inoculation of a large number of these mice, subsequent inoculation of the tumors which were obtained into other mice of the same breed was attended with a greater number of successful results. The "virulence" of the tumor might thus be said to be increased.

As regards the immunizing effect of unsuccessful inoculations but little has been determined in this investigation. Thirty-nine mice in which the Jensen tumor material was absorbed after its first inoculation, on reinoculation with the same strain of tumor, furnish negative results in thirty-one, and growths which attained large size in eight. This percentage (twenty per cent) of growths following the reinoculation of mice in which the tumor material had been absorbed after the first inoculation is considerably lower than the percentage (45.6 per cent) of growths following the inoculation of unused mice. In all cases the amount of tumor tissue injected was small, and in a number of instances a long interval of time separated the two inoculations. It does not appear that the absorption of tumor tissue in small amounts, such as were injected, is sufficient to produce any marked degree of immunity.

Three mice first inoculated subcutaneously with an adenocystoma of the lung, which was absorbed without any growth, were later inoculated with Jensen's tumor, which grew in all three.

Five mice that had previously been inoculated subcutaneously with mouse kidney tissue were later inoculated with the Jensen tumor; large growths resulted in two of the five cases.

Of five mice inoculated with the Jensen tumor, after having absorbed subcutaneously inoculated placental tissue, one developed a large growth.

In order to obtain reliable results in the production of artificial immunity to the inoculable tumors, it is necessary to work with mice which are known to be susceptible, otherwise it cannot be determined whether the immunity is natural or acquired. It is also necessary to use, for the immunizing injections, tumor material which is incapable of growth. With this in view, Ehrlich has used a tumor which grows in a small percentage of mice, and both Michaelis and Clowes have treated the tumor tissue with chemicals when injecting for immunizing purposes. In the above manner Ehrlich is able not only to protect mice against subsequent inoculations with the same tumor, but also against inoculations of tumors of another type. Since definite results have already been reached in this line of investigation, I have, thus far, undertaken no extensive experimentation relative to immunization against the inoculable tumors.

II. Inoculations with Ehrlich's tumor "Stamm 11."

This tumor has been carried for ten transplants. The percentage of successful inoculations (52.62 per cent) was slightly greater than was the case with the Jensen tumor. Notwithstanding that so much depends upon the susceptibility of the mice employed and the conditions of the inoculation, this tumor furnished more uniform results than the Jensen tumor. There is usually a gradual increase in the mass of the inoculated tumor tissue, and the tumors rarely make their appearance later than two weeks after inoculation. It is also very unusual for a tumor of this strain to disappear spontaneously after a period of unquestionable growth. Except in mice partially immunized by previous injections, this has occurred in but one instance.

The Ehrlich "Stamm 11" tumor usually shows more variation in structure than the Jensen tumor. In certain portions of tumors of this strain the epithelium is often arranged in solid masses, while in others it is gland-like in its arrangement. The structure of the growth, even in the more gland-like portion, is very irregular, and the spaces which correspond to the lumina of the glands often appear as irregular clefts showing that the epithelium is somewhat papillary in arrangement. As the tumors increase in size after inoculation into normal animals, the central portion usually becomes necrotic, and in large tumors there is often only a relatively thin layer of living tumor epithelium situated at the surface. The necrosis appears to be brought about by the failure of the supply of nourishment. The tumors of this strain are occasionally cystic, the cavities being filled with thin, slightly brownish fluid.

The rate of growth is fairly uniform, and there is much less individual variation.

The reinoculation of five mice, which had absorbed the tumor material previously injected, furnished actively-growing tumors in two, a tumor which grew for a time but later became absorbed in one, and no tumors in the remaining two.

An attempt was made to produce an immunity to the Ehrlich tumor by injections of the necrotic portion of tumors of the same strain. The dry, central portion of a large tumor was frozen and ground repeatedly until reduced to a homogeneous paste. Two large drops of this were injected subcutaneously into each of four mice. Six days later each mouse received a second dose of the same material. They were all inoculated with living tumor tissue fourteen days after the first protective injection. Of the four mice, two developed large tumors, indicating that immunity had not followed the injection of the necrotic tumor material.

Double inoculations with the Ehrlich tumor were tried in two instances. In one case the second inoculation was made fourteen days after the first. Tumors developed from each inoculation. In the other case the second inoculation was made forty-seven days after the first, from which a large tumor had grown. A nodule appeared at the site of the second inoculation, being about the size of a pea, three weeks later, and then diminished in size during a period of four weeks, and ultimately disappeared. The tumor resulting from the first inoculation, in the meantime, grew constantly.

Ten mice which had absorbed Jensen tumor material were reinoculated with the "Stamm 11" tumor. Six of these developed tumors, two of which ceased to grow, and were later entirely absorbed. In the other four mice the inoculation resulted negatively.

These experiments, as well as similar inoculations with the Jensen tumor, demonstrate that the absorption of small amounts of tumor tissue is often insufficient to protect an animal against subsequent inoculations of tumor tissue.

In the three mice received from Professor Ehrlich, the "Stamm 11" tumor was growing subcutaneously, and in one of these there were metastases in one of the lungs and in the liver. The presence of a nodule of the tumor in the latter organ is perhaps best accounted for by the growth of tumor epithelium from the lung nodule, which was of large size, into a pulmonary vein through which channel it could pass into the systemic circulation. In the liver the tumor is necrotic except a thin peripheral layer of living epithelium. The liver tissue where it comes in contact with the surface of the tumor is, for the most part, normal. At one point, however, there is a small group of necrotic liver cells. There is no flattening of cells or distortion of the normal structure of the liver suggestive of the exertion of pressure by the growth of the tumor, but the normal tissue seems gradually to have disappeared before the growth of the tumor epithelium. The amount of necrosis in the tumor is probably due to its failure to derive blood vessels of sufficient size for its nutrition from this organ.

Only one other case with metastases occurred in fifty-nine mice in which this strain of tumor grew on subcutaneous inoculation. In this instance the mouse was killed sixty-four days after inoculation. The metastatic nodules were situated in the lungs.

SUMMARY.

In carrying on experimental inoculations with the Jensen tumor, it has been kept up for ten generations or transplants. Actively-growing tumors developed in seventy-three (45.6

per cent) of the normal mice inoculated, and in the others the inoculations resulted negatively. Eleven (sixteen per cent) of the tumors developed for a time, but later disappeared spontaneously. The reinoculation of mice in which the first inoculation had resulted negatively produced tumors in only twenty per cent. Some of the tumors of this strain appeared soon after inoculation, others appeared after a considerable interval of time. One tumor was not apparent until sixty-four days after inoculation. Many of the tumors, which appeared late, afterward diminished in size and disappeared, but one which appeared sixty days after inoculation attained great size. From the result of the inoculation in the latter instance, it is evident that it is possible for the growth of the tumor epithelium to be suspended for a long period of time and then to assume great activity. It would be important to determine whether such fluctuations are due to some inherent property of the tumor cells or to the character of the soil in which they grow.

The Ehrlich "Stamm 11" tumor has also been carried through ten transplants. Actively-growing tumors appeared in fifty-nine (or 52.6 per cent) of the normal mice inoculated. In only one was there spontaneous retrogression. Tumors of this strain show greater regularity of growth than the Jensen tumors. They appear soon after inoculation and their subsequent development is usually quite uniform.

The tumors of both the above strains are often largely necrotic. This is apparently due to the unrestricted growth of the epithelium irrespective of blood supply. The growth of the tumor is so great that it outstrips the growth of its blood vessels, and the cells which are farthest from the supply of nutrition become necrotic.

Certain breeds of mice were found to be more susceptible to the two strains of inoculable tumor than others, and the inoculation of mice from one source always resulted negatively.

Metastases were present in four of the seventy-three mice in which the Jensen tumor developed. These were all situated in the lung. Various stages in the growth of these metastases were presented, and the various sizes of the nodules correspond quite closely to the interval of time between the inoculation and the death of each animal. Metastases were also found in the lungs of one and in the lungs and liver of another of the mice inoculated with the Ehrlich tumor. The manner of dissemination of the tumor cells in all these cases is usually quite obvious from the constant relation of the metastatic nodules to the pulmonary arteries. The nodules always arise from tumor cells which have lodged in the branches of the pulmonary artery, and, if sections are stained in a way to bring out the elastic tissue, the relation of this vessel to the nodule can be clearly distinguished.

In no instance were the lymph nodes adjacent to the tumor involved, although in many they were in actual contact with the surface of the tumor. In this respect these inoculated tumors behave quite differently from the spontaneous tumors of the mouse, which not infrequently invade the lymph nodes.

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DESCRIPTION OF PLATES.

PLATE V.

FIG. 1. — Tumor material situated beneath the skin of a mouse taken eight days after inoculation with the Jensen tumor. The greater portion of the injected tissue is necrotic, but the deeply stained layer at the surface of the mass consists of rapidly-growing tumor epithelium.

FIG. 2. — Metastases in the lung of a mouse killed forty-nine days after inoculation with the Jensen tumor. The tumor nodules, on account of their growth from the pulmonary arteries, are invariably grouped about the bronchi.

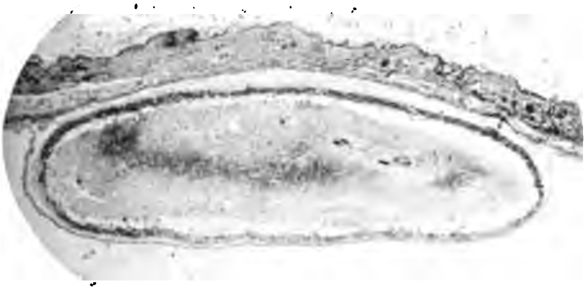
FIG. 3. — A section of a subcutaneous nodule of the Jensen tumor. The epithelium is arranged in alveoli, and portions farthest from the blood supply are necrotic.

PLATE VI.

FIG. 4. — Three small metastatic nodules in the lung of a mouse killed twenty-eight days after inoculation with the Jensen tumor.

FIG. 5. — Metastases of the Jensen tumor taken forty-one days after inoculation. The tumor epithelium has proliferated extensively within the pulmonary artery and is growing through its wall into the surrounding tissue.

FIG. 6. — From a section stained with Weigert's elastic tissue stain. The tumor tissue after occluding the artery has grown through its wall at one point. The wall of the vessel is outlined by elastic tissue.



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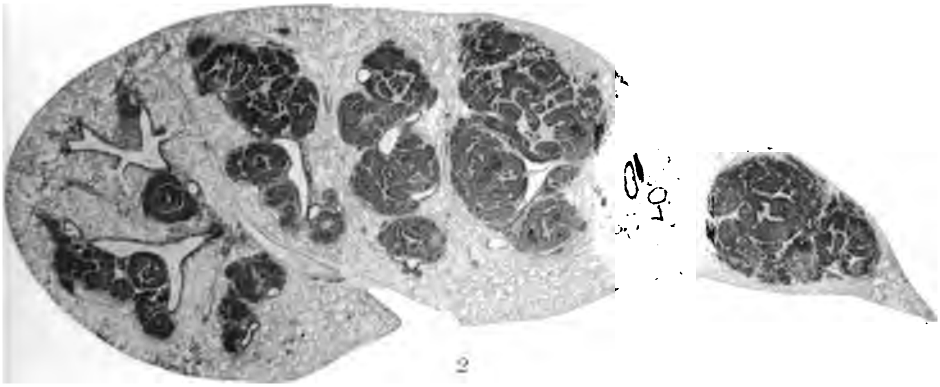

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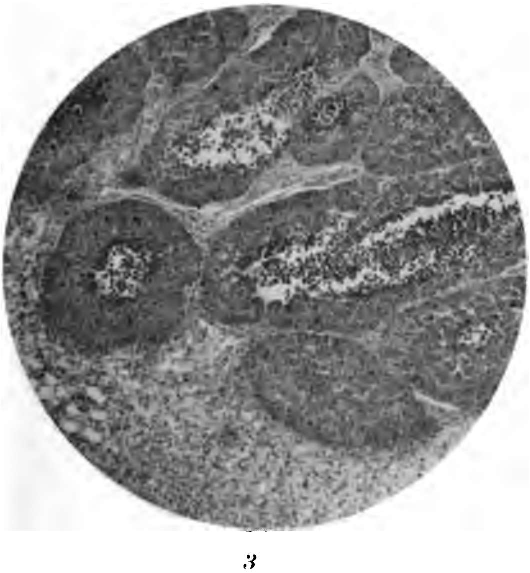

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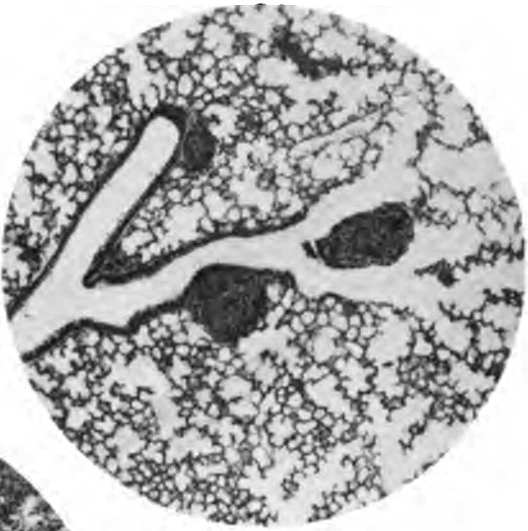

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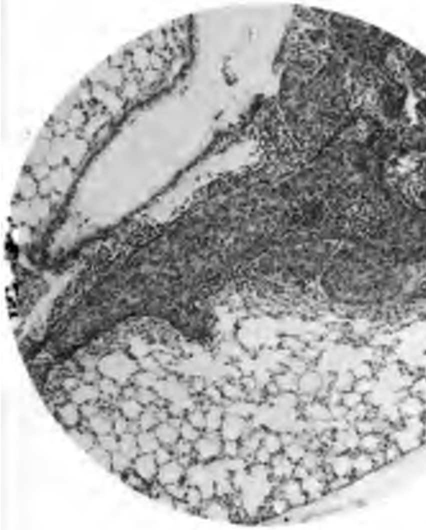

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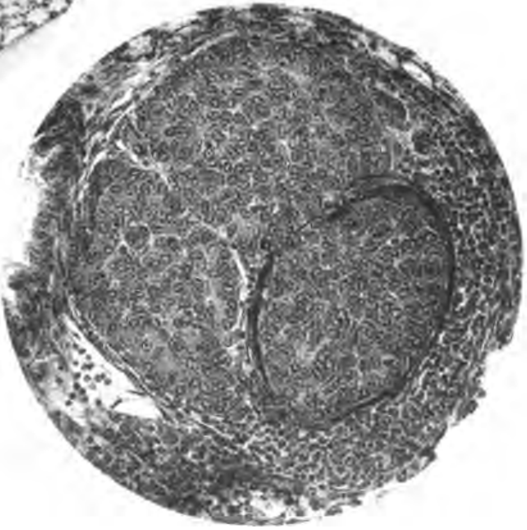

6

A SERIES OF TWENTY SPONTANEOUS TUMORS IN MICE, WITH THE ACCOMPANYING PATHOLOGICAL CHANGES AND THE RESULTS OF THE INOCULATION OF CERTAIN OF THESE TUMORS INTO NORMAL MICE.*

E. E. Tyzzer.

In uninoculated mice, kept for breeding purposes, a number of tumors have appeared spontaneously. Also in a series of mice inoculated with tumor tissue, certain ones have presented primary tumors in portions of the body at a distance from the inoculation site. Since nearly all of the primary tumors which occurred in inoculated mice developed in the lung, it was impossible to determine whether they were present previous to inoculation or developed after it. Several mice, in which there were actively-growing inoculated tumors, presented also primary tumors of the lung. Other mice, in which the inoculated tumors either failed to develop, or developed for a short time and later disappeared, were found to have small tumors in the lung at the time of their death. One mouse inoculated subcutaneously on the back with the Jensen tumor presented subsequently no evidence of growth at the site of the inoculation, but a nodule appeared four weeks later in the mammary gland which proved to be a primary tumor. All these tumors are of distinctly different type from those used in the inoculations, and they undoubtedly represent true primary growths.

Since the various primary tumors have occurred with about equal frequency in both the inoculated and the noninoculated mice, there is no evidence that the inoculations stand in any causal relation to their development. Until such relation is shown, all primary tumors are regarded as spontaneous rather than experimental.

In the series of cases presented in this paper, the object is to give the most important points in the case-history, the pathological changes in the various organs, and the results

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of the inoculation of normal mice with the tumor tissue, as well as descriptions of the various primary tumors which have been met with.

1. Papillary cyst-adenoma of the lung.

Livingood[6] has described a tumor of the lung which occurred in a white mouse dying of an experimental bacterial infection. This tumor presented the appearance of an adeno-carcinoma, and seemed to originate from a bronchus. Within the bronchus it was found growing as a papilloma, at one point breaking through its wall, and growing into the surrounding tissue. He states that the greater portion of the tumor is made up of infiltrating fingers of epithelial cells.

Haaland[5] found nodules in the mouse's lung arising by the proliferation of the alveolar epithelium. Since these nodules were found in mice previously inoculated with tumor material, he suggests that they may have resulted from the presence of the inoculated tumor, but points out the necessity of distinguishing such nodules from the true metastases which originate with tumor cells that have lodged in the blood vessels.

Primary tumors of the lung have been found in twelve of the mice which have been under observation. In only one case did a tumor of this type attain sufficient size to interfere with the function of the lung. In the other cases the primary tumors appeared as minute nodules none of which exceeded four millimeters in diameter, so that their true nature was not apparent until sections were made.

Case No. 1.[*] — A black female mouse, apparently quite old, died three months after its purchase. This mouse was kept apart for breeding purposes, and was never inoculated. An autopsy was made immediately after death.

A grayish white mass measuring 5 x 10 x 10 millimeters is found situated in the superior lobe of the right lung. The surface of the mass, presenting to the lateral wall of the thorax, has a slightly depressed central portion and an elevated border. There are several smaller nodules in the same lobe and two very minute nodules in the middle lobe of the right lung. The kidneys present a puckered, uneven surface with many minute clear spots, evidently retention cysts, and scattered throughout the substance of the organ are many opaque whitish or yellowish points. Near the anterior end of the right kidney is a yellowish white nodule, one and a half millimeters in diameter, elevating the surface. The other organs present no gross lesions.

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[*] Full report of case in The Journal of the American Medical Association, 1906, XLVII., 1237.

A fresh teased preparation of the mass in the lung examined microscopically showed it to be an epithelial growth. Portions of this were placed in fixatives for histological study and the remainder inoculated into five mice.

The primary tumor of the lung has an alveolar structure and consists of epithelium which lines spaces made very irregular by projecting folds and processes of connective tissue. Different portions of the tumor vary considerably in structure. In certain portions the epithelial cells are much larger than in others. The central portion of the tumor has degenerated, and is largely replaced by connective tissue. There are no mitotic figures to be found, but direct nuclear fission is a prominent feature. Many of the nuclei present eosin-staining inclusions, which are often surrounded by a thin layer of deeply-staining material so that it resembles, somewhat, the nuclear membrane. The supporting tissue of the tumor is relatively small in amount and consists of both fibrous and elastic tissue, the latter element in relatively large amount. It is apparent that the tumor increases both by internal growth, — expansion — and by the growth of epithelium outward over the surfaces of the alveoli. The smaller tumor nodules are of similar structure. There is no evidence of metastasis by way of the blood vessels or lymphatics. It is possible that the dissemination of detached cells through the air spaces has been the mode of origin of the smaller nodules.

Certain portions of the lung about the tumor are atalectatic and it is here difficult to distinguish the exact limits of the latter. The alveolar walls are thickened and the lining epithelium is more or less cuboidal in type. Masses of cells filling the air spaces of the lung, both in portions adjacent to the tumor nodules already described and in portions at a distance from them, are difficult to interpret. These cells vary in size, but average much smaller than the epithelial cells of the tumor nodules. They also differ from the latter in that they stain less intensely and possess relatively small spherical nuclei. Whereas the epithelium in the definite tumor nodules is invariably found covering surfaces in a single layer, these cells, on the other hand, always tend to fill spaces.

They resemble very closely the swollen epithelial cells of the alveoli and it is by no means certain that they are not identical. The amount and arrangement of this epithelium is, however, very suggestive of a new growth (Fig. 5). In certain portions of the lung the normal structure is replaced by an irregular reticulum of connective and elastic tissue, in the interstices of which are masses of epithelial cells of this type. In the denser portions of this reticulum the epithelial cells are much flattened and distorted, while in the larger spaces they are polygonal. A few of these cells were found in mitotic division. In some instances they attain great size, and are then often multinucleated. Whether or not the masses of epithelial cells filling spaces in the lung represent a diffuse tumor growth, the condition certainly indicates a great proliferative activity throughout the lung.

The nodule situated in the kidney of this mouse consists of irregularly arranged epithelium with staining affinities closely resembling those of the convoluted tubules. The nodule is sharply defined and distinct from the kidney substance. It extends through the entire thickness of the cortex and bulges slightly from the surface of the kidney. The epithelium composing the tumor is columnar and in places is apparently ciliated. In portions of the nodule the epithelium has a tubular arrangement, while in other portions the growth is very irregular. Extending about a portion of the nodule there is a flattened cavity, making it appear probable that the growth has originated from the epithelium lining a cyst. Near the surface of the nodule is a mass of eosin-staining material consisting chiefly of dead epithelial cells. There are several long, pointed crystals, probably cholesterin crystals, in this material. The cortex of the kidney is much thinner than normal, and scattered throughout its substance are cysts of various sizes lined with swollen epithelium. Many of the tubules contain casts, and there are interstitial changes with collections of lymphoid cells and increase of connective tissue.

A portion of the lung tumor was snipped into small bits and inoculated subcutaneously into five mice. Following the inoculation a small mass appeared in each mouse at the site of inoculation. This was apparently due to exudation about the material inoculated, and a fortnight later it had entirely disappeared. In one mouse, sixteen days after the inoculation, there was a minute subcutaneous nodule which increased slowly in size and appeared as a rounded, slightly elevated translucent mass about three millimeters in diameter. Four weeks after its appearance this mass, which was evidently a small cyst, ruptured and practically disappeared, but after a few days it began to fill out again.

After rupturing a second time it finally disappeared about fourteen weeks after the inoculation. All five mice were under observation for the remainder of their lives, but nothing developed at the site of this inoculation. One and a half months after the inoculation with lung tumor three of the five mice, which were of a single litter, were inoculated with the Jensen tumor. In two of the mice this tumor grew rapidly and caused their death within two months after its inoculation. In the third mouse the growth of the Jensen tumor was very slow, and for long periods showed no apparent increase in size. This mouse, when killed, presented, in addition to the inoculated Jensen tumor, a small primary tumor of the lung. This is taken as Case No. 2 of this series.

The mouse, in which a small cyst had developed after inoculation with the lung tumor, died at the age of a year and five months and presented no new growth. The other mouse lived for eleven months after the inoculation with the lung tumor and developed no new growth.

Case No. 2. — A young gray male. This mouse, when about a month old, was inoculated subcutaneously on the back with bits of the lung tumor of Case No. 1. As nothing appeared at the site of inoculation, the mouse was again inoculated about one and a half months later with the Jensen tumor. A month after this inoculation, a minute nodule appeared beneath the skin of the back and this was soon followed by a second nodule. These nodules grew very slowly, and at times were apparently stationary for weeks.

The mouse was killed accidentally when five and a half months old, and was autopsied sixteen hours after death. The inoculation of the two nodules of Jensen tumor into other mice was followed by growth in several cases.

In the posterior surface of the superior lobe of the left lung near its lower border there was a sharply circumscribed, grayish white nodule, about one and a half millimeters in diameter. It seemed probable that this nodule represented a metastasis of the inoculated tumor, but on study of sections it was found to be a tumor of another type. The other organs appeared normal.

In serial sections, the tumor nodule appears to be of the form of a broad based cone, the base of which is at the pleural surface and the apex of which is in close relation to a terminal bronchus. The tumor consists of epithelium situated upon irregular folds and processes of connective tissue. The epithelium consists of rather large cuboidal cells, arranged in a single layer, and possessing relatively small spherical nuclei. The cell-outlines are not prominent, but are distinguishable. No mitotic figures are found. The interstices of the tumor and the alveoli about it are filled with material, apparently a coagulum, in the form of delicately reticulated spheres such as are often seen with secreting cells.

Blood vessels extend into the tumor from various sides. The pleura covering the tumor is much thickened, and the elastic tissue is here present in a large amount. There is also a large amount of elastic tissue in the folds and processes upon which the epithelium is situated (Fig. 6). There is a tiny adhesion at the border of the lung, but no pathological process is found elsewhere.

In this case the tumor of the lung was of the same type as that used in the first inoculation, but, with the data at hand, it cannot be inferred that this tumor resulted from either of the two inoculations. Since it is of a type quite distinct from the Jensen tumor, which was growing subcutaneously in this animal, it cannot be regarded as a metastasis.

Furthermore, by its relations to the surrounding structure of the lung, it is shown to be a primary tumor.

Case No. 3 (1321). — A white male, which was used for breeding purposes, died at the age of one year and two months. The superior lobe of the right lung presents near its anterior border a translucent whitish nodule about one millimeter in diameter. This nodule consists of epithelium covering irregular folds of connective tissue, and is similar in many respects to those found in the two preceding cases. The central portion of the nodule is, however, almost solid, and here the epithelial cells are small and enmeshed in a reticulum of connective tissue. At the periphery of the nodule the epithelial cells are larger and are arranged somewhat irregularly so that they do not always form a single layer of cells. The supporting structure of the folds is so delicate that it requires special staining methods to bring it out clearly. A small bronchus terminates against this tumor, and the accompanying artery, which is not especially enlarged, ramifies through its structure. A few polynuclear leucocytes are present, scattered through the tumor, and in the surrounding lung tissue there are many large phagocytic cells filled with pigment. In another lobe of the lung is a minute focus of inflammation adjacent to which there is a parasite situated in the lymphatic vessel about one of the branches of the pulmonary artery. In section this parasite presents a body wall with short spines projecting from its outer surface. Within this is a group of bodies having the general appearance of ova. From both ends of each of these there project several small spines, and each contains nuclear material which is in some divided into few, in others into many separate masses.* The liver and spleen present no lesions of importance. The kidney is of normal size, and, considering the age of the animal, is in good condition. There are a few casts of blue-staining granular material within the tubules, and several dilated tubules. There is a small number of parasites, Klossiella muris, present and the remains of several worms encapsulated in dense fibrous tissue.

Case No. 4 (1301). — A white female mouse, one of the offspring of Mouse No. 3. This animal, when slightly over five months old, was inoculated with Ehrlich's tumor "Stamm 11." There was a slough at the site of the inoculation, but the tumor grew, nevertheless, and attained large size, killing the mouse about nine weeks after the inoculation. The central portion of the inoculated tumor was at this time necrotic. There was a minute whitish spot in the outer surface of the superior lobe of the left lung. In sections this is found to be a very minute tumor nodule, measuring .6 millimeter in diameter, and of the same type as was found in the preceding cases. Elastic tissue forms a considerable proportion of the supporting tissue of the tumor.

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* Borrel[1] has called attention to a worm which he observed in the lung of a mouse which possessed a tumor.

In the liver there are collections of cells, chiefly lymphoid and plasma cells, about the portal canals. The kidney contains large numbers of Klossiella muris and there are extensive interstitial foci. Many of the glomeruli are injected, and the epithelium of Bowman's capsule is thickened in many instances.

Case No. 5 (1385). — A white female mouse, offspring of Mouse No. 3. This mouse was never inoculated. It died at the age of one year, three and a half weeks. A small tumor nodule was found in the middle lobe of right lung. The tissues were somewhat decomposed, but sections of the nodule show the tumor to be of the usual type.

Case No. 6 (1353). — A white male mouse, offspring of Mouse No. 3. Inoculated when two and a half months old with bits of a lympho-sarcoma of Mouse No. 11. Following this nothing appeared at the site of inoculation. This mouse always appeared pale and sickly. Died at the age of seven and a half months. A small tumor nodule was found at the inferior border of the superior lobe of the left lung. The folds and processes of this tumor, which was of the usual type, are somewhat compressed so that the nodule appears almost solid. A large dilated vein extends directly through this nodule. In the liver there is slight degeneration of parenchyma about the central veins. The kidney presents a proliferation in the epithelium of Bowman's capsules and several acute interstitial foci. There is also a slight infection with Klossiella muris. The spleen is small and presents no striking pathological process.

Case No. 7 (1544). — A black female mouse. This animal had never been inoculated. It died at the age of nine and a half months. The tumor in this case appeared as a minute translucent area, less than one millimeter across, situated in the outer surface of the superior lobe of the left lung. It corresponds to the usual type of lung tumors in mice, but in one portion the epithelium is composed of cells much larger than elsewhere and with an almost glandular arrangement. In a complete series of sections the tumor is not found to come in contact at any point with a bronchus. This is due to its flattened shape and its superficial situation at the surface of the lung.

Case No. 8 (1413). — Gray female. When four and a half months old this mouse was inoculated with the Jensen tumor. Two nodules appeared at the site of inoculation within four weeks after the inoculation, increased in size during the following two weeks, but afterward diminished in size and were absorbed in a short time. The mouse died at the age of ten and a half months, and presented a whitish, rounded nodule, two millimeters in diameter, near the lower border of the superior lobe of the right lung. This is found to be a tumor similar to those described in the preceding cases. Certain portions of the growth are, however, papillary in type. Elastic tissue is found in greatest amount in the older portions of the tumor. It occurs chiefly in the connective tissue septa, but is practically absent from the papillary portions of the tumor. In several places it has grown into bronchi where it retains its papillary type (Fig. 8). The interstices of the tumor, as well as the surrounding lung tissue, present material in the form of either fine granular precipitate or delicate rings.

Amyloid is found in the liver, spleen, and kidney. There is nephritis attended with extensive loss of cortical substance. In those areas where the process has been most severe, the tubules contain casts of deeply-staining material, and similar material is often found in a layer covering the glomerular tufts. Klossiella muris is present in large numbers.

Case No. 9 (1412). — A gray female mouse, born in the same litter with Mouse No. 8. Inoculated subcutaneously with the Jensen tumor when four and a half months old. One week after the inoculation there was a minute mass beneath the skin. This disappeared, and nothing further developed at the site of inoculation. The mouse died when eleven months old. Several grayish spots were noted in the lungs when fresh, but after fixation in Zenker's fluid a very minute nodule was found near the lower border of the superior lobe of the left lung. When sectioned this nodule is found to be a tumor of the type of the primary tumors of the lung previously found in other mice There is a large blood vessel extending through this tumor, but at no point is there any bronchus in contact with it.

Three other tumors of this type were found in addition to primary tumors of the mammary gland, in Cases Nos. 13, 15, and 16, which are given in full further on. The nodule in Mouse No. 13 is very minute and was discovered accidentally in stained sections of the lung. It is situated on a surface of a lobe which is in apposition to the surface of the lobe below. At one point the nodule unites with a bronchus. The tumor is composed of irregular folds covered with epithelium, but it does not form so definite a nodule as in the preceding cases. In Case No. 15 the nodule was apparent at the surface of the lung, but extends far into the lung where it has grown into a bronchus. The lung tumor of Case No. 16 is the only one in which mitotic figures have been found.

All the tumors of this series correspond to a single type although there are minor variations. They have thus far, in every instance, been situated at the surface of the lung, and are covered externally by pleura. They consist of epithelium situated upon irregular folds and processes of supporting tissue in which there is a relatively large amount of elastic tissue. There is invariably an increase in the elastic tissue of the pleura covering the surface of the tumor.

In some instances the plications of the tumor are separated by wide spaces so that there is considerable resemblance to the cystic new growths; in other instances the structure appears compressed and solid and the epithelial cells are situated in the meshes of a reticulum of connective tissue. Different portions of these tumors show great variation in structure and the epithelium may have either a gland-like or a papillary arrangement. It is a difficult matter to decide what name to apply to this type of tumor. It is not invariably glandular in structure, neither papillary nor cystic, yet for want of a better term it is designated "papillary cystadenoma."

The minute size of most of these growths makes it possible to determine quite accurately the site of their origin. They seem to bear no constant relation to the bronchi, although the latter often terminate at their surface. In Case No. 7 the tumor nodule is of such minute size and so situated that it is wholly independent of the bronchi. The relation of the tumor to the pleura seems to be quite constant, and, in all, the oldest portion of the tumor appears to be near the surface of the lung. The arteries from which these nodules derive their blood supply are branches of the pulmonary artery, and are often of considerable size.

The growth of most of these tumors is probably slow. Mitoses were found in only one instance. In many of the cases the tissue was not fixed until after the animal had been dead for some time, but in Cases Nos. 13, 15, and 16 the tissue was fixed immediately after killing, and in Mouse No. 1 soon after death. Mitotic figures were found only in the lung tumor of Case No. 16. On account of the rarity of indirect cell-division, it might be thought that these nodules were congenital and remained stationary throughout life. In one case, however, a growth of this sort was found filling a large portion of the right thorax, the existence of which throughout the life of the individual would be impossible. The growth of this tumor appears to have taken place in two ways — by internal increase through which the whole mass expanded, and by the growth of the epithelium over the surfaces of the surrounding alveoli, a process which is more or less analogous to the infiltrating growth of tumors outside the lung. In the same case there were also several smaller nodules of tumor in other portions of the lung, some of which are situated in other lobes. These smaller nodules did not occur in such relation to the blood vessels as to suggest a vascular dissemination of the tumor tissue. In Mouse No. 5 there were several small tumors situated in different lobes of the lung. It is possible that in these instances there were multiple primary tumors, or the smaller nodules may have originated with tumor cells which had been separated from the primary growth and distributed by way of the air passages. In two of the cases, the tumor had extended into the bronchi, so that its malignant character is unquestionable.

A tendency of the epithelial cells to degenerate is apparent in all the tumors of this type. Many cells contain two or more nuclei evidently resulting from direct fission. The nuclei may attain great size, are often irregular, and contain large hyaline inclusions. The retrograde changes are greatest in the central portion of the tumor and here the epithelium may be, to a large extent, replaced by connective tissue.

In Case No. 1 the lung presents, in addition to the definite tumor nodules, masses of cells filling the air spaces.

The cells which comprise them resemble very closely swollen cells of the alveolar epithelium. They differ from the epithelial cells of the definite tumor nodules in that they tend to fill spaces, whereas the latter always cover surfaces. Mitotic figures are occasionally found in these cells, but never in the epithelium of the more definite nodules in this case. Attention has been called in a previous paper * to the possible identity of these masses of cells and certain nodules which Haaland[5] has described as tumors arising from the alveolar epithelium in the lungs of mice previously inoculated subcutaneously with tumor material. I am now convinced that such is not the case, but that the nodules which he describes are, as he regards them, true adenomata.

It is possible that these masses of cells represent a diffuse form of tumor, but most of the evidence indicates a general proliferative activity on the part of the alveolar epithelium,

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* Jour. of the American Medical Association, 1906, XLVII., 1240.

possibly of the nature of a reaction to the primary tumor, which in this case attained a large size. It is not uncommon to find similar cells in chronic and acute processes in the lung. The cells in the present instance occasionally contain crystals, but show slight evidence of phagocytic activity.

Nine of the mice presenting lung tumors were females, and three were males. The age of these animals was not extremely great. The youngest was somewhat over five months, and the oldest, of known age, a year and two months. Six of the mice which presented tumors of this type had been previously inoculated, and six had never been inoculated with tumor tissue. It may be noted that the youngest mice which possessed these tumors were mice which had been inoculated. This may be explained by the fact that the chances for life are much greater in uninoculated than in the inoculated aninals.

Whether the injection of tumor tissue into the body of certain animals may in some way serve to excite certain tissues to irregular growth is a question upon which the present data throw no light. The possible influence of heredity in the development of these tumors will be discussed in another paper.

Minute tumors such as those presented in this series are easily overlooked, and it is probable that they are more common than is generally supposed. One tumor was not apparent at the time of the autopsy, but was discovered accidentally in a stained section. When small nodules are found in the lungs of mice which have tumors elsewhere, it is quite natural to conclude that they represent metastases. That such a conclusion may be erroneous is obvious from the occurrence, in certain of the above cases, of primary growths in the lungs of animals in which there was an inoculated tumor growing subcutaneously. If a histological study be made, there will be no difficulty in distinguishing a primary growth in the lung from a metastasis on account of the constant relation of the latter to the pulmonary artery or to the lymphatics.

2. Cyst-adenoma of the kidney.

Nodules were found in the kidneys of two mice. A description of one of these has already been given in Case No. 1, in which there was also a tumor of the lung.

Case No. 10 (1146). — A yellowish female, apparently quite old when purchased. This mouse was kept with the breeding mice, but had only one litter of three young. It always displayed a voracious appetite and devoured great quantities of food, but nevertheless remained rather thin. It lived for six months subsequent to its purchase, and was finally killed on being found moribund. There was a whitish nodule measuring two and a half millimeters in diameter in the posterior extremity of the left kidney a short distance from the hilus. The liver was small, and appeared to be less than one-fourth the normal volume for a mouse of this size. The lobes were rounded and somewhat constricted in places so that portions of the organ were pedunculated. The spleen was also of small size.

The nodule found in the kidney consists of a mass of irregularly arranged epithelium projecting into a cyst causing the latter to appear crescentic in section. The cyst is lined with epithelium, which is irregularly columnar in places, cuboidal or flattened in others, and forms a continuous, unbroken layer over the surface of the invaginating mass. The surface of the epithelium lining the cyst is everywhere uneven and groups of swollen, vacuolated cells form rounded, villus-like projections. The invaginating mass consists of epithelium similar to that lining the cyst, but is characteristically tubular in arrangement. It is also vacuolated as though it had contained fat droplets. The tubular arrangement is very irregular and in many places there are solid masses of epithelium. Several small cysts are found in the other parts of the kidney. There are small numbers of Klossiella muris present but not in the nodule.

In the liver the portal veins are dilated. There is degeneration and necrosis of scattered cells, usually most numerous in the central portions of the lobules. The capsule is much thickened at the points of constriction.

Groups of disintegrating cells are found in the spleen.

These nodules of the mouse's kidney appear to arise by the irregular proliferation of the epithelium lining cysts into the cavities of the cysts. Cysts in which the lining epithelium forms processes and folds which project into the cavity probably represent an early stage in the formation of these nodules (Fig. 10). The epithelium in the larger nodules has assumed an irregular tubular arrangement (Fig. 11).

In Case No. 10 the epithelial proliferation appears to have taken place outside the cyst and formed a mass which projects into the cavity of the cyst, but presses the cyst wall before it.

In neither of the two cases did the nodule present any evidence of active growth. No mitotic figures were found, but it is impossible to draw any conclusions as to the limitations of growth in these nodules. There is often considerable regenerative activity shown in the tubular epithelium of the kidney of the mouse. This is seen in cases of nephritis in which large portions of the kidney parenchyma are destroyed. It is possible that the nodules just described represent some such activity, although, in the second case, there was no destruction of kidney substance. Occurring together with a cyst-adenoma of the kidney there was, in Case No. 1, a primary tumor of the lung with other more general changes throughout the organ in addition to an advanced nephritis, and in Case No. 2 an atrophy of the liver which was apparently effected by a gradual destruction of liver cells.

3. Lympho-sarcoma.

Haaland[5] has described a malignant lymphoma in the mouse. In his six cases there was a great increase in the lymphoid tissue throughout the entire body. He, however, considered this increase to be of the nature of a general hypertrophy or hyperplasia of preëxisting tissues rather than of the nature of a neoplasm. Inoculations gave negative results, but, after placing normal mice in the cage with the mouse having a lymphoma, five similar tumors appeared in the space of two years.

The following two cases differ from Haaland's cases in that they present a well defined primary growth without appreciable hyperplasia of lymphoid tissue elsewhere in the body.

Case No. 11 (1350). — A yellowish brown female, which lived a year and a month after its purchase. It raised during this time five litters of young. When the last litter was well grown, a soft, spherical nodule, slightly over one centimeter in diameter, was found in the right inguinal region. This increased in size during the following period of ten days, at the end of which it was removed by operation. It consisted of a sharply circumscribed, homogeneous mass, the tissue of which appeared rather translucent with a pinkish tinge. Portions of the tumor were placed in various fixing fluids and the remainder used in the subcutaneous inoculation of seventeen mice. The mouse appeared weak after the operation, and, when seven days later breathing became labored, it was killed and autopsied at once. The operation wound appeared clean, but had not healed. The lungs present ill-defined grayish areas. The liver appears normal. The spleen is small and dark red. The posterior extremity of the left kidney is occupied by a large cyst, one centimeter in diameter, and filled with clear fluid. The left inguinal and axillary lymph nodes are small. In the mesentery is a mass of lymphoid tissue, five millimeters in length, appearing reddish and mottled so that it resembles somewhat splenic tissue. The skin is thin, and easily torn.

The rounded mass which was found growing in the inguinal region consists chiefly of small round cells. It contains very little connective tissue, but is permeated by a network of small blood vessels and capillaries, which show the large nuclei and spur-like processes characteristic of growing blood vessels. It is slightly less vascular in its center than near the surface. The cells, of which the mass is chiefly composed, are of the type found in the germinative centers of lymph follicles. The nuclei are considerably larger and more vesicular than the nuclei of lymphocytes of the type most commonly found in the blood. The cytoplasm is indefinite in character, and the cell outline is indistinct so that it can only be made out in certain areas of the section. Usually the cytoplasm of these cells appears as an indefinite, loosely reticulated material lying between the nuclei. Mitotic figures are numerous, and these, so far as can be ascertained, are of the normal type. The tumor mass is homogeneous throughout and there is no lobulation or tendency toward arrangement in follicles. Large phagocytic cells are numerous in certain areas near its surface. Many of the perivascular lymph spaces are filled with small lymphocytes, which are much smaller than any of the cells found elsewhere in the tumor. Portions of this tumor were impregnated with silver, following the technic of Levaditti,* in order to determine if spirochetæ were present. None of these organisms were found.

Most of the lymph nodes of this mouse, which was killed one week after the removal of the tumor, are small and present no prominent germinative areas. The lymph nodes are composed chiefly of cells of the plasma-cell type; that is, they possess a small rounded nucleus with the chromatin in dense masses and a relatively large amount of cytoplasm which stains bluish or lilac, and presents a definite cell contour. Mitotic figures are numerous. Many of these cells are vacuolated, others are filled with hyaline globules, and in places there are many disintegrated cells. The large mass of lymphoid tissue found in the mesentery appears to be a greatly enlarged lymph node. The sinuses contain blood, and the lymphatic cords present large numbers of polynuclear leucocytes. There are many giant cells such as those found in the bone-marrow and spleen.

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* Levaditti, C. L'histologie pathologique da la syphilis héréditaire. Anal. de l'Inst. Pasteur, 1906, XX., No. 1.

There is an active leucocytogenic bone-marrow.

Portions of the lung contain large numbers of irregularly rounded, lilac-staining bodies, possibly of the nature of corpora amylacea. There are also irregular, laminated deposits of material resembling fibrin in its arrangement situated in the lung tissue and long, slender crystals in the bronchi. These portions of the lung are filled with large phagocytic cells many of which contain greenish pigment or small crystals. There are many collections of lymphoid and plasma cells in the pleura and several fibrous adhesions.

The liver presents collections of cells of the plasma-cell type about the portal canals, but no other lesion.

The spleen contains large numbers of cells of the plasma-cell type and mitotic figures are numerous. The organ contains only a small amount of blood.

The large cyst present in the left kidney is lined with thin, flattened epithelium. There are many hyaline casts and cystic dilatations in the tubules in both kidneys. Many of the glomeruli are filled with hyaline material presumably amyloid. Klossiella muris is present in small numbers.

The mammary gland is filled with secretion and appears normal foi this stage of its activity.

Nothing appeared at the sight of the inoculation in any of the seventeen mice inoculated subcutaneously with this tumor. The injected material could not be found in one mouse of this series which died seventeen days after the inoculation, so that it must have been absorbed at once. Nearly all of these mice lived for several months. One mouse of this series presented a minute tumor of the lung (see Case No. 6).

In this case there is in the inguinal region a definite primary tumor composed of tissue resembling lymphoid tissue. Since no inguinal lymph node was found in its vicinity it seems not improbable that it has replaced the latter structure. The tumor mass consists of small cells similar to those which compose the germinative areas of lymph follicles, a system of small blood vessels permeating its whole structure, and a relatively small amount of connective tissue most of which is situated about the blood vessels. Certain portions of the tumor contain numerous phagocytic cells. The tumor is limited by a delicate capsule of connective tissue, but none of the surrounding tissue was removed at operation, so that it was not determined whether this was invaded. Since the mouse died one week after the operation, nothing could be determined concerning recurrence.

The study of the various organs and tissues reveals no evidence of the dissemination of the tumor into other parts of the body. There is a chronic pleuritis and a chronic process in the lung associated with calcareous deposits. There is also a slight nephritis with what is probably amyloid deposited in the glomeruli. The lymphoid tissue throughout the body, although not increased, is somewhat modified, and cell differentiation appears to be greatest towards the type of the plasma cell. A lymph node of the mesentery is the only one which shows any appreciable increase in size. This is infiltrated with leucocytes and contains hemorrhage as well.

The inoculation of this lympho-sarcoma into seventeen normal mice resulted negatively, although several were the offspring of the mouse in which the tumor developed.

Case No. 12 (1715). — A black male about five and a half months old. Derived by mating a member of Family A (see section on Heredity), with a mouse of the Bashford strain. Died suddenly after a few convulsive movements.

There is a large mass situated in the thorax just anterior to the heart and apparently originating in the mediastinum. It is pale pink in color, and is slightly lobulated, although its surface is in general smooth. It measures 1.4 x .9 x .5 centimeters. There are numerous smaller nodules about the roots of the lungs and in the pericardium. Minute elevations are apparent on the surface of the lung. There is an escape of blood as the thorax is opened.

The tumor was not sectioned until after it had been fixed in Zenker's fluid. There then appeared to be softened areas in its interior.

Histologically the tumor is of the type of that described in the preceding case. It consists of small cells similar to those of the germinative centers of the lymph follicles, and possesses scarcely any stroma. The tumor is quite vascular and there are beautiful examples of growing blood vessels. In certain places there is hemorrhage into the tissue, in other places there are large numbers of disintegrating tumor cells and large phagocytes loaded with cell detritus. Mitotic figures are very numerous. The nodules observed in the pericardium consist of tissue similar in every respect to that found in the large tumor mass.

The tumor is found growing rather diffusely in the pleura, sometimes in thin strands, at other times forming irregular nodules. All the large arteries and veins of the lung are surrounded by tumor. The walls of the alveoli are somewhat thickened in certain portions of the lung. One portion of the lung presents a chronic inflammation with large numbers of phagocytic cells filled with eosin-staining needle-shaped crystals.

There is no hyperplasia of the lymphoid organs elsewhere in the body. None of the other organs presents any important lesion. The liver appears practically normal. In the spleen there is a marked degree of erythrocytogenesis. The stomach and the kidney contain small numbers of the usual parasites. The pancreas and brain appear normal.

The lympho-sarcoma originating in the mediastinum of this mouse possesses a structure practically identical with that of the tumor of the preceding case. The tumor in the present instance, however, had produced metastases in the pericardium, and had extended along the lymphatics accompanying the large vessels throughout the lung. Judging from the number of mitotic figures present, its growth was rapid. There are minute hemorrhages into its substance in places. In the interior of the mass there are many disintegrating tumor cells, and these are being taken up by the large phagocytes which are present.

4. Subcutaneous adeno-carcinoma.

Of the neoplasms which develop in mice, the epithelial tumors originating in the subcutaneous tissue have received most attention. By series of inoculations many have been artificially propagated for long periods of time. It is probable that many of these tumors originate in the mammary gland. They usually arise on the under surface of the body or on the sides, and occur most frequently in females, especially in old females which have been kept for breeding purposes. Various types of epithelial tumors occur.

Michaelis[8] has distinguished three types of epithelial tumors: 1. Cancer, the epithelium of which is arranged in alveoli, with little or much connective tissue according to the age of the growth. The Jensen tumor is an example of this type. 2. Adeno-carcinomata, which have a glandular structure, and present ducts and alveoli with regular cubical epithelium. Certain tumors of this group show a tendency to cyst formation through the dilatation of the glands. Others have a papillary structure through the uneven growth of the gland epithelium. 3. Tumors, the epithelium of which is divided by the connective tissue into large alveoli. In gross appearance these resemble tumors of the first type. Each alveolus is, however, subdivided into smaller groups.

Haaland[5] has described four types of epithelial tumors: 1. Adeno-carcinomata (tumors of the Parisian type). Metastasis takes place by way of the blood vessels. Neighboring lymph nodes are not invaded. 2. Tumors of the Jensen type. 3. Epidermoid carcinomata occurring on the jaw and invading the neighboring lymph nodes. 4. A molluscoid tumor, with lobules arranged as radiating rays with rounded bases at the periphery, from its structure possibly related to the hair follicles.

The most complete classification of epithelial tumors is given by Apolant.[1] The epithelial tumors of subcutaneous origin are divided into two groups, adenomata and carcinomata. With these two groups he distinguishes the following types:

1. Adenoma simplex. These are encapsulated, solid tumors having a lobular structure. The alveoli correspond very closely with those of the normal mammary gland, but are closely packed. In certain respects they resemble goitre. In the youngest tumors there is often no sharp demarcation between tumor and mammary gland. These structures appear to arise by hypertrophy of portions of the mammary gland.

2. Cyst-adenoma simplex. Such tumors are essentially similar to the preceding, the only difference being found in the dilatation of the lumina of the glands by secretion. This fluid may appear normal or more serous than normal.

3. Adenoma cysticum edematosum. In tumors of this type there is degeneration of the connective tissue between the alveoli, and the epithelium lining the cysts may also become degenerated, making the interpretation of the growth difficult. An additional factor is found, in some instances, in the condition of the blood vessels. The capillaries may

be so dilated that the structure resembles a cavernoma. Hemorrhage may occur producing adenoma cysticum hemorrhagicum.

4. Cyst-adenoma papillare. In instances in which the proliferation of the epithelium exceeds the cyst formation, papillæ are formed. The stroma may be small in amount or abundant.

1. Carcinoma simplex alveolare. In many adenomata the epithelium, in certain places, proliferates and grows wherever it finds space. Carcinoma may develop from the hemorrhagic cystadenomata. These tumors (cystocarcinoma hemorrhagicum) present great irregularity in structure and are usually poor in stroma. They form a large percentage of all mouse tumors, and are relatively benign.

2. Carcinoma papillare, including the " spalten bildendes " carcinoma. These tumors arise from the papillary cyst-adenomata.

Apolant states that whereas, in man the transition between adenoma and carcinoma is rare, in the mouse it is frequently observed.

Loeb[7] has described a tumor in a Japanese mouse, which presented two varieties of epithelium — cubical epithelium forming alveoli filled with colloid material, and epithelium possessing finely or coarsely vacuolated cytoplasm. The latter cells disintegrate to form spaces filled with fluid. The structure of this tumor suggests some relationship to the salivary or sebaceous glands.

The tumors presented in the following four cases are all of different types. They resemble quite closely mouse tumors described by other authors.

Case No. 13 (1415). — A black and white female, of breed obtained from Dr. E. F. Bashford. This mouse, when about ten months old, was inoculated subcutaneously on the back with Jensen tumor tissue. Nothing appeared subsequently at the site of the injection. Four weeks after the inoculation the left axillary lymph node was enlarged, and, one week later, a small nodule in the left inguinal region and a larger rounded oblong mass behind the right axilla were discovered. The enlarged left axillary gland later on appeared spherical and translucent, and had evidently become cystic. The mass behind the right axilla subsequently became harder, but showed increase in size. The mouse was killed about four weeks after the enlargement of the lymph nodes was first noted.

In the left axilla is a cyst filled with thin, clear, pale yellow fluid. In the mammary tissue, posterior to right fore-leg, is a flat nodular mass, measuring about one centimeter in length, and of whitish color, except one portion which is dull pink and resembles lymphoid tissue. Bits of this mass, examined microscopically, showed an epithelial tumor, and a portion was accordingly used to inoculate ten normal mice. Extending from this mass towards the axillary lymph node is a whitish cord of tissue evidently an obstructed lymphatic vessel. The mammary gland about the mass presents many opaque whitish granules. The lymph nodes about the lower jaw are greatly enlarged, probably due to inflammation about the lower incisors. The pancreas appears as a rounded nodule attached to the mesentery. The uterus contains several embryos measuring eight or nine millimeters in length and several smaller and probably abnormally developed ova. The histological findings are as follows:

The cyst, which was situated in the left axilla, is found to consist of a lymph node distended with clear fluid in which are several lymphoid cells and polynuclear leucocytes.*

Sections of the nodule from the right axilla show a large collection of lymphoid cells surrounding a mass of carcinoma tissue.

The lymphoid cells are intermingled with fat cells and mammary gland.

The included tumor consists of epithelium lining several large spaces, which are filled with eosin-staining, granular precipitate, and desquamated and necrotic tumor cells. The tumor epithelium is very irregular, but in many places shows a gland-like arrangement. Many of the degenerating cells are flattened and squamous in character, and, where they are compressed in small spaces, appear as epithelial pearls (Fig. 16).

In these the cells at the center stain deeply with eosin, while the surrounding cells contain keratohyaline granules, and resemble the cells of the stratum granulosum of the epidermis. Mitotic figures are not numerous in the tumor epithelium and certain portions are wholly necrotic and surrounded by giant cells. There is an area of marked edema in the connective tissue near the tumor, and several mammary gland ducts, in the same area, are dilated. The surrounding tissue is very vascular. Polynuclear leucocytes are nowhere present in large numbers, so that the reaction of the tissue appears to be much of the nature of the reaction about a foreign body.

The cord of tissue extending from the mass just described toward the axilla consists of foreign-body giant cells and lymphoid cells surrounding necrotic tumor epithelium. In places there are collections of polynuclear leucocytes, and the whole is very vascular.

The nodule situated in the left inguinal region is found to be a small primary tumor, of which the various portions differ greatly in character. It is situated against the inguinal lymph node which indents it slightly so that it is more or less cup-shaped.

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* Cystic nodules have occurred quite frequently among the mice under observation. They appear as swellings beneath the skin and have in several instances been mistaken for tumors.

The tumor consists, for the most part, of closely packed tubular glands. In some portions the glands are of uniform type, but in other portions the epithelium, instead of being arranged in a single layer, is greatly thickened and grows irregularly. In the central portion of the tumor, the epithelium is growing in large solid masses and presents all the characteristics of the carcinoma. Glands dilated with fluid form cysts in various parts of the tumor. Mitotic figures are numerous throughout the tumor, but are somewhat less numerous in parts showing the uniform glandular structure than in parts where the epithelium is growing in solid masses. A delicate network of connective tissue forms the stroma of the tumor. The adjacent inguinal lymph node is not involved.

A very minute tumor, which was overlooked at the time of the autopsy, is apparent in sections of the right lung. It measures about .8 millimeter and is situated in the lung substance and against the pleura in that portion of its surface which is in apposition with the surface of the adjoining lobe. It is of the same type as the other primary tumors of the lung, described in the preceding series, and it is distinct from the tumor of the breast which was found in this case, so that it cannot be considered a metastasis of the latter. The lymphatics around many of the branches of the pulmonary artery are filled with lymphoid cells.

The portal veins of the liver are greatly dilated. There are a few small collections of cells of the lymphoid and plasma-cell type about the portal canals and scattered elsewhere.

The spleen, which is large, shows erythrocytogenesis in a marked degree. Areas corresponding with the lymph-follicles are composed of tissue such as composes the germinative centers and this is also found distributed rather diffusely through the organ. Leucocytogenesis is nowhere in evidence. Disintegrating cells are found in moderate numbers. The pancreas appears normal.

The kidney contains a small number of Klossiella muris, but, except for a few minute acute interstitial foci, is otherwise normal.

The lymph nodes throughout the body show considerable activity and the germinative areas form a prominent feature. The submaxillary lymph nodes present the greatest degree of hyperplasia probably on account of an inflammation about the lower incisors.

The mammary gland appears normal and active.

Ten mice were inoculated subcutaneously on the back with bits of tissue from the mass situated posterior to the right fore-leg. In all of these there was ulceration and sloughing at the site of the inoculation. The tumor failed to develop in any of the inoculated mice. The mass from which the inoculations were made was later found to be a metastasis surrounded by a large amount of inflammatory tissue.

In this case a primary adeno-carcinoma (carcinoma simplex alveolare), situated in the inguinal region, with metastasis to the axillary region of the opposite side, and a papillary cyst-adenoma of the lung were found in a female mouse, aged ten months, a short time after it was inoculated with the Jensen tumor. The primary tumor situated in the inguinal region is undoubtedly of mammary origin. A portion of the tumor is composed of glandular tissue of the mammary gland type, but this is quite distinct from the normal gland. While portions of this tumor present the structure of a simple adenoma, other portions consist of epithelium growing irregularly after the manner of a carcinoma, so that transition from adenoma to carcinoma is seen in many places.

A mass of tumor tissue occurring in the axillary region, where it is invading the lymphatics, is considered to be a metastasis of the primary adeno-carcinoma of the inguinal region, although certain features which it presents are not shown in the latter. The inflammation about this metastatic nodule and also the degree of degeneration which its epithelium presents possibly account for its failure to grow when inoculated into other normal mice.

A minute primary tumor was found in the lung of this mouse. That this does not represent a metastasis from the mammary tumor is shown both by its distinct structural characteristics and by its relation to the tissue of the lung. It is similar to the other papillary cyst-adenoma of the lung already described.

The inoculation of this mouse with the Jensen tumor a short time prior to the finding of these tumors is another factor to be considered. There can be no question, however, as to the primary character of both the tumor of the lung and the adeno-carcinoma of the inguinal region. The tumor in the axillary region had grown into the lymphatics and showed also peculiarities, such as epithelial pearls, which have not as yet been observed in the Jensen tumor. Furthermore, it appeared at some distance from the site of the inoculation.

Except for the inflammation about the lower incisor teeth and a more or less general hyperplasia of the lymphatic tissue, there were no important changes in the other organs.

Case No. 14 (1489). — A full grown Japanese waltzing mouse.* This mouse presented a rounded mass, 1.5 centimeters in diameter, hanging from the neck just anterior to the fore-legs. The mass increased somewhat in size in the short period for which it was under observation. The mouse died from the shock of an operation for the removal of the mass. The gross appearance of the mass is as follows: It is covered with skin which is somewhat reddened and devoid of hair, but there is no ulceration. It is freely movable, and has a rounded slightly nodular surface. The tissue of which it is composed is light pink, rather soft, and, in addition to numerous minute cysts in its substance, there is a large cavity in its interior filled with a grayish, milky fluid.

The adjacent right axillary lymph nodes are somewhat enlarged, and there is a whitish line of tissue extending posteriorly into the mammary gland, and just posterior to this is a minute translucent nodule not over a millimeter in diameter. At the site of the left inguinal lymph node there is a small, sharply circumscribed nodule measuring 3 x 2 x 2 millimeters. In the right horn of the uterus is a minute translucent area about the size of a pin head. In the superior surface of the liver are many minute yellowish points.

The tumor consists of epithelium some of which is distinctly glandular in its arrangement. The lumina of the glands usually contain an eosin-staining, colloid material. Scattered through the tumor are vacuoles suggesting the presence of fat. Many of the tumor epithelial cells are filled with small vacuoles and such cells occur in nests, so that the structure of the sebaceous glands are closely simulated. In portions of the tumor the epithelial cells are flattened and spindle-shaped, so that they resemble the cells of a sarcoma. There is very little connective tissue in the structure of the tumor, but this is in places intimately intermingled with the epithelium. In certain areas the stroma presents large numbers of compound granule cells, with a peculiar form of degeneration. The nuclei appear hollow and the chromatin is arranged about the nuclear membrane. In the clear space within the nucleus is usually one or more masses of eosin-staining, hyaline material. In the stroma and surrounding tissue there are also small numbers of plasma cells, which are also markedly degenerated. The silver impregnation method of Levaditti brings out small numbers of spirochetæ, confined chiefly to the hyaline secretion and the cysts filled with broken down material. Mitotic figures are found in considerable numbers, but are not so numerous as in the inoculable mouse tumors.

The adjacent axillary lymph nodes are not invaded with this tumor.

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* Presented by Dr. Ernest Leroy Hunt, of Worcester, Mass.

The nodule of the left inguinal region consists of tumor structure similar to that just described, and it undoubtedly represents a metastasis of the latter. In the center of this nodule there is a compact mass of tumor surrounded by a thin capsule of connective tissue. Outside of this central mass the growth of the tumor is less dense and presents many small cystic cavities. This portion of the nodule presents a striking resemblance to the preputial gland. In this region the stroma presents large numbers of compound granule cells, many plasma cells, and relatively few eosinophiles and polynuclear leucocytes.

There is a small amount of hyaline material about the hair follicles, against the lower surface of the epidermis, and about the mammary glands. With the different stains, the hyaline material is colored similar to connective tissue.

The lung presents no pathological process.

The spleen contains a large amount of blood and considerable golden yellow pigment, situated within phagocytic cells. It contains many erythroblasts and developing polynuclear leucocytes. There is, in places, considerable disintegration of cells, especially of lymphoid and plasma cells. Many of the plasma cells are vacuolated, and many are filled with large hyaline globules. The lymph nodes contain well defined germinative centers, but, apart from these they appear to consist chiefly of plasma cells in various stages of degeneration.

The wall of the uterus contains a collection of cysts lined with cuboidal epithelium differing somewhat from the epithelium of the endometrium. These cysts are situated partly within and partly external to the internal layer of smooth muscle. In other portions of the uterus there are small collections of lymphoid and plasma cells, and large phagocytic cells with golden yellow pigment.

The primary adeno-carcinoma of the neck was inoculated subcutaneously into four normal Japanese waltzing mice. One of these was killed accidentally nine days after inoculation. The injected material, in this case, is represented by a mass measuring 3 x 5 millimeters, and consists of epithelium intermingled with connective tissue and newly-formed blood vessels without any definite structural arrangement. Mitoses were present in about the same frequency as they occurred in the original tumor. Within this mass is one large and several smaller collections of necrotic tissue which takes for the most part a pinkish stain.

A second mouse died forty-five days after inoculation. Beneath the skin there was a group of small, rounded, translucent nodules, the largest of which was about three millimeters in diameter. Portions of these nodules are glandular in structure and resemble the original tumor. Groups of vacuolated epithelial cells, resembling the sebaceous glands, are also found. In one portion many cells of the tumor epithelium appear small and compressed, and there is here and there evidence of degeneration.

In the third mouse the injected tumor material increased slightly in size, but since then has remained practically stationary for six and a half months subsequent to the inoculation and is now under observation.

In the fourth mouse the injected tumor material increased slowly, but eventually produced a group of nodules of considerable size. A histological study showed a tumor epithelium growing in the lymphatics at some distance from the larger nodules. After growing for three and a half months this tumor was successfully inoculated into other Japanese mice. Several common mice similarly inoculated furnished negative results.

The adeno-carcinoma of this case is of a peculiar type, in that groups of the epithelial cells become vacuolated so that the structure of the sebaceous and preputial glands is closely simulated. Fat is found in considerable quantity in these portions of the tumor, and also as numerous minute globules in portions where the epithelial cells are compressed and spindle-shaped.

The epithelium also presents a true glandular arrangement, and the lumen of the gland is usually filled with homogeneous, hyaline or colloid material. In portions of the tumor the epithelial cells are compressed and spindle-shaped, and intermingled with the connective tissue. Mitotic figures are not rare, but are not so numerous as in the Jensen tumor. The adjacent lymph nodes are not invaded, but the inguinal node is replaced by a tumor nodule similar in every respect to the primary tumor.

The predominating cells in the lymph nodes are cells of the plasma-cell type, and these show various degrees of degeneration.

The wall of one horn of the uterus contains a group of cysts (Fig. 12). It is impossible to determine whether this represents a congenital anomaly or a more recent development. The epithelium differs from that of the endometrium. Mitotic figures are not infrequent.

The inoculation of the adeno-carcinoma of this case into four normal waltzing mice has resulted in a slow but well defined growth in two, and further inoculations have been successful. One was killed accidentally nine days after inoculation. In the other, which died forty-five days after inoculation, the tumor epithelium was alive.

This tumor resembles very closely the one found by Loeb[7] in a Japanese waltzing mouse. He succeeded in inoculating other mice with this tumor, but certain of the tumors resulting from these inoculations were of a mixed type consisting in part of epithelium, in part of sarcoma tissue. The results obtained were therefore similar to those of Ehrlich,[3,4] who by the inoculation of epithelial tumors produced at first mixed tumors containing both epithelial and sarcoma tissue, but which, on further inoculations, furnished a certain number of pure sarcomata in which there remained no epithelial tissue. Both Ehrlich and Loeb believed that the sarcoma element was not derived from the original primary tumor, but arose from the proliferation of the connective tissue of the inoculated animals.

Case No. 15 (1559). — A brown female mouse, exact age unknown, but evidently quite old. Four and a half months after its purchase a hard nodular mass was discovered behind left fore-leg. It was killed with chloroform and an autopsy made at once.

The skin is thin and tears readily. A mass situated in the mammary tissue behind the left fore-leg measures 1.4 x 1 x .5 centimeters. This is for the most part slightly translucent white in color, but on section more opaque grayish streaks and markings are apparent within it. It is hard and elastic — somewhat of the consistency of cartilage. Posteriorly this mass thins out, but it is otherwise rounded. By frozen sections it is found to be an epithelial tumor. A smaller mass of tumor tissue is situated just anterior to that already described, and another small mass is situated beneath the pectoral muscles. Both of these masses represent lymph nodes replaced by tumor. In the posterior surface of the superior lobe of the left lung is a white nodule about two millimeters across, and somewhat nearer the apex is a small adherent area. The cortex of the bones is much thicker than normal. The long bones such as those of the legs are hard, and the marrow-space is no thicker than a very fine cambric needle. The spleen is large and of dull, light red color. The other organs present no macroscopic lesions.

The mammary tumor was inoculated subcutaneously into eight normal mice. Hard nodules appeared in two of the mice inoculated, and increased steadily in size. In a third mouse there is a small mass which has not as yet shown any appreciable growth. Sufficient time has not elapsed to determine the results of further inoculation.

In the skin of this mouse the hair follicles and sebaceous glands are surrounded by faintly-staining, hyaline material, staining blue with Mallory's connective tissue stain. In places the material is found lying against the inner surface of the epidermis, and it surrounds nearly all the blood vessels of the skin, capillaries included. In places it appears as a continuous layer of hyaline, in others it has the form of very thick wavy fibers. The mammary glands are, to a large extent, replaced by this hyaline material, and are represented by irregular branching cords of hyaline in which there is a core of crowded epithelial cells intermingled with which is yellowish pigment.

The tumor is composed of epithelium, the arrangement of which is peculiar in that in it are everywhere found irregular clefts. From this it is apparent that there is a tendency on the part of the tumor epithelium to irregular papillary ingrowth.

The stroma is small in amount, but is apparently dense. A large portion of the primary growth is necrotic, and the living and dead tissue is intimately intermingled. The epithelial cells are somewhat smaller than those of the Jensen tumor, and the cell-outline is indistinct. The nuclei are vesicular, and, as they are relatively large, appear closely packed in the tumor epithelium. Mitotic figures are everywhere numerous. The epithelium, although in places it occurs in rather large solid masses, tends usually to form surfaces within its substance. It is seldom that the surfaces bound cylindrical spaces as is the case with the lumina of glands, but the surfaces almost invariably bound very irregular clefts in the epithelium, so that it has an irregular papillary arrangement. The tumor has infiltrated the overlying skin and comes into contact with the epidermis. There is a small ulceration at one point. The fibers of the panniculus carnosus involved in the tumor present various degrees of degeneration. The tumor epithelium has grown along a large nerve which is situated beneath it, and is also found in a subcutaneous lymphatic at a distance from the border of the tumor.

The mass situated just anterior to the primary tumor consists of tumor tissue which has almost completely replaced a lymph node. The peripheral sinus is outlined by a layer of tumor epithelium, and there is a small amount of lymphoid tissue in one portion of the nodule. The central portion of the mass presents a large amount of necrotic tumor material. On one side, the tumor has infiltrated the connective tissue. The structure of the tumor is here identical in every respect with that of the primary growth. Relative to the amount of necrosis present there is but little inflammatory reaction.

The next lymph node situated beneath the pectoral muscle presents a more recent involvement with the tumor. Portions of its structure still remain and the follicles are readily distinguished. The peripheral sinus is on one side filled with a solid mold of tumor epithelium, but there is at one point an accumulation of lymph, slightly dilating the capsule of the lymph node. There are also other smaller dilatations of the lymphatics evidently due to obstruction by the masses of tumor epithelium. As compared with the primary growth, there is here a relatively small amount of necrotic tissue.

The nodule in the lung is a tumor of another type from that of the mammary gland. It consists of cuboidal or columnar epithelium growing upon irregular folds or septa of connective tissue. The cytoplasm is much more abundant in this epithelium than in the mammary tumor and it stains more intensely with eosin. Several large blood vessels extend into its substance. Whereas the mammary tumor presented large numbers of mitotic figures, the epithelium of this tumor presents none.

The nuclei are often irregular and many contain peculiar hyaline inclusions such as have been found in other tumors of this type situated in the lung. Direct nuclear division is apparently common. Near the periphery of the nodule are several small collections of lymphoid cells. Other portions of the lung appear practically normal. At one point on the pleura there is a small mass of inflammatory tissue, probably of the nature of an adhesion.

In the liver, about the portal canals, are large collections of cells, most of which are of the leucocyte series. Intermingled with these are large cells filled with yellow pigment, with here and there delicate needle-shaped crystals. In the kidney there are numerous minute foci of acute interstitial nephritis. Many of the tubules contain casts. There is a moderately severe infection with Klossiella muris. Between the cortex and medulla of the adrenal there are several large groups of cells with small shrunken nuclei and abundant cytoplasm, which is filled with coarse irregular granules, and is somewhat vacuolated. The cells have a slightly greenish tinge and are probably phagocytes filled with some form of pigment.

The spleen is large and active, erythrocytogenesis and leucocytogenesis are marked. These processes are more prominent than is the formation of lymphoid cells. In certain areas there are cells loaded with pigment. There is no amyloid and nowhere any destructive process.

The lymph nodes, other than those invaded by tumor, are not notably enlarged, but present considerable activity. Besides the formation of cells of the lymphoid series there is, in all those studied, evidence of more or less leucocytogenesis. In many instances the sinuses are filled with large phagocytic cells, in the cytoplasm of which are found large numbers of slender needle-shaped crystals which stain with eosin. These cells also contain more or less yellowish pigment and occasionally portions of other cells. There is considerable disintegration of cells of the lymphoid and plasma-cell type.

The bone-marrow of the femur is reduced in amount. Its activity appears to be chiefly leucocytogenic, and erythroblasts are but rarely seen. There are many large cells with a pinkish-staining cytoplasm which contains masses of sharp-pointed, slender crystals, pigment, red blood corpuscles and leucocytes. The cortex of the bone is thick and stains unevenly.

The glands of the stomach are dilated and filled with large numbers of a unicellular organism, many of which are in the process of segmentation. They are invariably found in the secretion of the glands and never occur

within the cells. Certain ones are encapsulated. Beyond the dilatation of the peptic glands there is no reaction on the part of the tissue.

Both large and small intestines appear normal.

In this case there occurred in an old mouse, which had never been inoculated, a primary adeno-carcinoma ("spaltenbildendes carcinom") of the mammary gland with metastasis to the adjacent lymph nodes, and also a primary papillary cyst-adenoma of the lung. The mammary tumor in the gross appeared whitish in color and was hard and elastic. It is composed of epithelium which is irregularly papillary in arrangement, so that it forms irregular surfaces, and contains many cleft-like spaces. It is actively growing, infiltrates the surrounding fat and connective tissue, and is found growing in the subcutaneous lymphatics. The stroma of the tumor is small in amount, but appears very dense. A large portion of the tumor is necrotic. This tumor has the same structure and arrangement where it has invaded the lymph nodes. The same tendency to undergo necrosis is here also apparent. The tumor of the lung is of another type and is identical with other primary tumors which have been found in the lungs of mice. It presents no mitotic figures.

In addition to primary tumors of two distinct types, this mouse presents many other pathological changes. The skin, which appeared rather thin and friable in the gross, shows a peculiar process. The small blood vessels and capillaries of the skin are surrounded by faintly-staining, hyaline material. This is also deposited about the sebaceous glands and hair follicles and is found in places beneath the epidermis. This material has to a large extent replaced the mammary gland. The ramifications of the gland are outlined in hyaline, but there is usually found a central core of degenerating gland-epithelium, intermingled with which are small collections of yellow pigment.

The lymph nodes, liver, and spleen show marked leucocytogenic activity. The bone-marrow is much reduced by a general osteosclerosis, but other than the presence of phagocytic cells filled with needle-shaped crystals, it shows no important pathological process. The lymph nodes and spleen also contain large numbers of phagocytic cells filled with masses of slender, needle-shaped crystals. The kidney of this mouse shows, besides a slight nephritis, a severe infection with Klossiella muris. Another species of parasitic protozoan is found in large numbers in the dilated glands of the stomach.

Case No. 16 (1647). — A white female mouse of unknown age and never inoculated. Six and a half months after purchase a rounded, somewhat triangular mass was observed just anterior to right hind leg and extending upwards toward the back. This mass was removed by operation, and was found to be in close relation to the mammary gland at its lower angle. The mass measures 1.8 x 1 x .8 centimeters, and has an outer convex, slightly nodular, surface and a concave, smooth, inner surface where it lies against the body wall. In its outer surface are rounded blackish and dull reddish areas, some of which are very minute, others a millimeter or more in diameter. The consistency of the tumor is moderately firm, but its tissue is easily crushed into a homogeneous mush. It appears to be subdivided into rather coarse lobules. A portion of the tumor was fixed for histological study, and the remainder inoculated into ten normal mice. Nothing has developed as the result of these inoculations.

The tumor consists of epithelium showing everywhere a tendency to glandular arrangement. The dark coloration of certain portions of the tumor are due to hemorrhage into its substance. The tumor is subdivided by septa of connective tissue into lobules, many of which are relatively large. Different portions of the tumor vary greatly in structure. Certain lobules are cystic, and there is in some instances papillary ingrowth of the epithelium. In other portions dense connective tissue has apparently grown into the glandular tissue, and has replaced it to a large extent. In other portions of the tumor the glands are filled with dense colloid material which takes the eosin stain. In the large lobules of the tumor the epithelium has a distinct glandular arrangement, but there is very little connective tissue, and elastic tissue is practically absent within the lobules. The blood vessels are here of large size, but extremely thin walled. There are many large spaces in the tumor epithelium, and these spaces are often traversed by the thin walled blood vessels, and it is here that hemorrhage apparently occurs, being dependent upon the delicacy of the vessel walls and the looseness of the surrounding tissue. In the older hemorrhages the red blood corpuscles are to a large extent disintegrated, and their substance is being taken up by phagocytic cells. In places there are large numbers of pigmented cells in the connective tissue around such a hemorrhage. In the connective tissue there are, in places, collections of lymphoid and plasma cells and numerous eosinophiles. There are also many nongranular leucocytes. Mitotic figures are present in the tumor epithelium in moderate numbers.

The mouse in which this tumor developed recovered from the operation and remained well and vigorous for nearly two months after the operation. There was no evidence during life of recurrence at the site of operation. Following pregnancy the mouse appeared ill and the abdomen became asymetrically distended. On killing the mouse the distention is found to be due to a large collection of pus and necrotic material enclosed in a portion of one of the horns of the uterus. The abscess cavity measures 2.5 centimeters in diameter and contains a macerated fetus. All the other organs of the peritoneal cavity are bound up in adhesions. The liver appears larger than normal and has a dull brownish color. Spleen large, rounded, and dark red. The lower lobe of the right lung presents a translucent, whitish nodule three millimeters in diameter. There is no trace of the operation wound in the inguinal region, but near by there is a small nodule 2.5 millimeters in length.

The nodule which occurred near the site of operation in the inguinal region is similar histologically to certain portions of the original tumor previously excised. It is glandular in structure throughout, and presents considerable colloid material within the lumina of the glands. The nodule in the lung possesses a structure identical with that of the primary tumors of the lung already described. It contains a cyst about 1.5 millimeters in diameter. There are numerous eosin-staining bodies found in the cytoplasm of the tumor cells. Many of the nuclei also present eosin-staining hyaline bodies. Mitotic figures, many of which are atypical, are found in this tumor.

The capillaries of the lung contain great numbers of polynuclear leucocytes. The lungs also present a peculiar hyaline thickening of the intima of the pulmonary veins. The liver and spleen, as well as the bone marrow, show marked leucocytogenic activity.

In this mouse there are two primary tumors, one arising in the inguinal region, the other in the lung. The former is of the type described by Apolant as Cysto-carcinoma hemorrhagicum. Different portions of this tumor vary greatly in structure. Large portions of the tumor possess very little supporting tissue. The structure of the tumor is loose, and there are spaces within the lobules filled with fluid and in some cases possessing a very delicate reticulum. The blood vessels ramify through these spaces, and, since they are thin walled, hemorrhage readily takes place. Portions of this tumor present a more characteristic glandular structure and there is considerable eosin-staining, colloid material within the gland alveoli. In portions of the growth, where there is a peculiar intermingling of connective tissue with the glands, it appears that spaces occurring in loosely constructed epithelial tissue have become filled with proliferating connective tissue. Following the removal of this tumor recurrence took place, a small nodule two and a half millimeters in length being found two months after the operation.

The primary tumor of the lung is similar in structure to those already described in the preceding series. It is peculiar in that it presents mitotic figures in moderate numbers. These had, heretofore, never been observed in the primary tumors of the lung.

This mouse appeared well and vigorous for two months subsequent to the removal of the inguinal tumor, and became pregnant. It was killed on account of a septic condition complicating pregnancy.

Considering the various types of spontaneous tumors which have thus far developed, the papillary cyst-adenomata of the lung have been by far the most frequent in occurrence, although tumors of this type seem to have attracted but slight attention. These tumors, on account of their situation, are only found by careful post-mortem examinations and some owing to their minuteness may, even at that, be overlooked. They occur in males as well as females, often in individuals which are not of great age. Since mitotic figures have been found in only one of them, it is probable that their growth is usually relatively slow. On account of the nature of the organ in which they develop, they may be malignant. The lung may be almost entirely replaced by a tumor of this type. The tumor enlarges not only by internal expansion, but its epithelium also grows outward into the lung along the alveolar walls and sometimes grows into the bronchi. In this series all the tumors conform to one general type of structure, although there are minor variations even in different parts of the same tumor.

The cyst-adenoma occurring in the kidney also appears to be a slow-growing tumor. It seems probable that such growths originate by the irregular proliferation of the epithelium of cysts within the kidney. The tumor epithelium resembles somewhat that of the kidney tubules.

A group of cysts found in the uterus of one case may possibly represent a congenital anomaly, although this cannot be determined.

The lympho-sarcomata of this series are actively-growing tumors, and consist chiefly of cells similar to those found in the germinative centers of lymph follicles. The blood vessels present blind branches and spurs such as are found in the developing blood vessels of granulation tissue. Large phagocytic cells are found in certain portions of the tumor.

The four subcutaneous adeno-carcinomata are all of different types. In one case the primary tumor presents all the transitions between a simple adenoma and an active, irregularly growing, carcinoma. The tumor of the second case is peculiar in that groups of its epithelial cells appear vacuolated so that the structure of the sebaceous follicles is closely simulated. Other portions have a true glandular arrangement. In the third case the primary tumor is hard and elastic. The hardness of this tumor appears to be due to the great density rather than to the amount of its stroma. It is composed of epithelium having an irregular papillary arrangement so that there are irregular or crescentic clefts. There is a relatively large amount of necrosis. The fourth tumor, on account of its loose structure and the tendency of the epithelium to form spaces, shows a marked degree of hemorrhage.

As might be expected the tendency to metastasize is much greater in certain tumors than in others. Multiple nodules were found in certain of the cases of papillary cyst-adenoma of the lung, but it could not be determined whether these originated by transplantation of the tumor epithelium by the way of the air passages, or whether they represented multiple primary growths. In the cases of adenoma of the kidney there was no metastasis. In one of the cases of lympho-sarcoma there was no metastasis, but in the other there were metastases in the pericardium, pleura, and lungs. With three of the four subcutaneous adeno-carcinomata there was invasion of the lymphatic system. With the mammary tumor, which showed transition from a simple adenoma to a carcinoma, what was considered to be the metastasis was of a type somewhat unlike the primary growth. In the other two cases the tumor-tissue replacing the lymph nodes retained the structural arrangement of the primary growth.

There appears to be a difference between the spontaneous tumors and the Jensen and Ehrlich "Stamm 11" inoculable tumors, in that the former invade the lymphatic system whereas the latter similarly situated have never done so, but have metastasized by way of the blood stream. However, in a successful inoculation with the tumor of a Japanese waltzing mouse, Case No. 14, tumor epithelium is found growing in lymph vessels at some distance from the main mass of the tumor. It would also appear that metastasis is more frequent in the spontaneous than in the inoculated tumors, at least when the latter are situated subcutaneously.

The results of the inoculation of normal mice with six of the tumors of this series are as follows:

Tumor Inoculated.	No. of Inoculations.	No. of Results.	Positive Results.
No. 1. Cyst-adenoma of lung	5	5	0
No. 11. Lympho-sarcoma	17	17	0
No. 13. Carcinoma simplex (metastasis)	10	0 (Suppuration)	0
No. 14. Japanese carcinoma	4	2	2
No. 15. Papillary carcinoma	8	7	2
No. 16. Hemorrhagic carcinoma	10	10	0
6 tumors	54	41	4

Thus only two of the six tumors inoculated have been successfully propagated, and these two were subcutaneous adeno-carcinoma. The number of inoculations presented is not sufficiently great to make the determination of the percentage of positive results of value. It is to be noted, however, that whereas certain tumors grow readily on transplantation, other tumors fail to grow even when inoculated under the most favorable conditions, so that the inoculable tumors undoubtedly possess possibilities of growth not shown by other tumors.

Inasmuch as the epithelium of the inoculable tumor is able to remain alive under unfavorable conditions, such as freezing and subjection to various chemicals, it appears that it is its relatively great vitality which enables it to grow on various soils such as are found in the tissues of different individuals. The noninoculable tumors, on the other hand, appear to require some special condition for their growth, a condition not found generally in other individuals.

The pathological processes found in relation with spontaneous tumors may possibly be of the nature of senile changes, or they may represent coincident conditions. Nearly all the lesions found in mice with tumors occur also in mice with no tumors. Parasites of various types are frequently met with. Bacteria are found in the lesions of various organs. Spirochetæ are sometimes found within the primary tumors as well as in other tissues. The significance of their occurrence will be taken up in another paper.

Sarco-cystis muris in the voluntary muscles and Klossiella muris in the kidney are of common occurrence. Encysted worms have been found in the lung and in the kidney. As marked, an atrophy of the liver was found in mice without tumors, as was found in Case No. 10, which presented an adenoma of the kidney. Nephritis is so frequent in the mouse that no significance can be ascribed to its occurrence in mice with tumors. Amyloid is frequently found in the liver, spleen, kidneys, and adrenals, and less frequently in the intestine in mice which are cachectic from bacterial infection. In several mice with tumors there was a deposit of hyaline around the small blood vessels, hair follicles, and occasionally just beneath the epithelium of the skin. In one case this material was deposited about the acini of the mammary gland, which had atrophied to such an extent as to be scarcely recognizable. In the same case there was also a general increase in the thickness of all the bones.

Tumors in mice may interfere with the body functions, especially if organs such as the lungs or heart are involved. In such instances the mere mechanical action of the tumor may cause death. On the other hand, certain mice possessing external primary tumors of great size appear strong and vigorous. Thus, since there is not evidence to establish any causal relation, it seems probable that the pathological processes found in mice with tumors represent coincident conditions.

Concerning the frequency of tumors among mice the consideration of numbers is of little significance unless age is considered as well. The sixteen cases here recorded occurred in somewhat less than eight hundred mice which have been under observation during two years past. Of these about five hundred either have been killed or have died, and the others are now under observation. An autopsy is made on each mouse which dies, in order to determine the presence of internal as well as external tumors. The best possible conditions are afforded the mice kept under observation so that they may attain as great age as possible. In certain instances old mice have been purposely purchased, and it is among these that two of the four subcutaneous tumors have occurred. The infrequency of tumors in mice indicated in the reports of certain investigators who have had enormous numbers of these animals under observation may be possibly explained in the failure to enable them to attain sufficiently great age.

In the present series of cases, the frequency of occurrence of double primary tumors in mice is to be noted. In sixteen mice with primary tumors there was a second primary tumor of a different type in four (or twenty-five per cent). This does not include the case which presented a collection of cysts in the uterus in addition to a subcutaneous tumor. Thus, in a given number of mice possessing a primary tumor, the probability of occurrence of second primary tumors appears to be greater than is the probability of occurrence of primary tumors in an equal number of mice, of the same age, taken at random.

The fact, stated earlier in this paper, that certain of the primary tumors of this series occurred in mice previously inoculated with tumor tissue is worthy of comment. The question at once arises, "Are these primary tumors to be regarded as the effects of the inoculations?" Ehrlich[3,4] believes that, in certain instances, the inoculation of epithelial tumors into normal mice has in some way stimulated the connective tissue at the site of the inoculation to proliferate so that there resulted a mixed tumor, which on further transplantation resulted in a pure sarcoma. Loeb[7] also obtained in Japanese mice a growth of sarcoma by inoculating a carcinoma. Haaland[5] found nodules arising by the proliferation of the alveolar epithelium in the lung of a mouse which had been previously inoculated with the Jensen tumor. From this he suggests the possibility of metastasis of tumors without the actual transplantation of cells.

In twelve of the cases presented in this paper, primary, papillary cyst-adenomata of the lung were found in six uninoculated mice and also in six mice which had been previously inoculated with tumor tissue. In one of the latter the inoculation of Ehrlich's "Stamm 11" had resulted in a large actively-growing tumor; in another mouse a lympho-sarcoma, which had been inoculated, failed to grow as it did in all other cases; and in four mice, inoculated with the Jensen tumor, there was in one a slow-growing tumor, in one a mass which developed at the site of inoculation, but later disappeared spontaneously, and in two no growth at the site of inoculation. In one of the latter there was, in addition to the primary tumor of the lung, a primary growth in the inguinal region which appeared soon after the inoculation. In all of these cases it is possible that the primary tumors of the lung may have been present when the inoculations were made. It is of course impossible to determine this point on account of the situation of these tumors. In the case presenting the mammary tumor, two nodules, one representing the primary growth, the other the metastasis, were first noted five weeks after the inoculation. The short period intervening between the inoculation and the appearance of this tumor with its metastasis makes the hypothesis untenable that it developed as the result of the inoculation. Since in a long series of inoculations with the Jensen tumor there has been no similar instance, it seems reasonable to regard the occurrence of a primary tumor, following an inoculation, as an example of coincidence rather than cause and effect.

When inoculated and uninoculated mice are compared as regards the frequency of occurrence of primary tumors, it is of interest to note that, in those kept under observation, primary tumors are more frequent in the uninoculated. This is possibly explained by the fact that a large number of the inoculated mice die at a comparatively early age as the result of the tumors which develop, and this number probably includes those mice which are most susceptible to tumor growth. Only those in which the inoculation results negatively live to attain old age, and many of them must be regarded as immune to tumor growth.

SUMMARY.

1. Papillary cyst-adenomata of the lung have been found in twelve mice. These tumors consist of epithelium covering thin folds and processes of supporting tissue in which there is a relatively large amount of elastic tissue. Since mitotic figures were found in only one tumor of this type, they are probably of slow growth, but may attain such size as to interfere with the function of the lung, and sometimes grow into the bronchi.

2. Cyst-adenomata of the kidney such as were found in two cases probably arise by the irregular growth of the epithelium lining the cysts. They show no evidence of active growth, and are probably benign.

3. Two lympho-sarcomata, one arising in the inguinal region of a female, the other in the mediastinum of a male

mouse, consist chiefly of cells resembling those found in the germinative centers of the lymph follicles. They contain little supporting tissue, but are permeated with small actively-growing blood vessels. Phagocytic cells are numerous in some portions of these tumors. These tumors appear to be of the nature of definitely localized primary growths. In one case, other portions of the body were not involved, in the other there were metastases in the pericardium, pleura, and lungs.

4. Subcutaneous adeno-carcinomata occurring in four female mice, are all of different types. Metastasis had occurred in three of these cases. In the fourth animal recurrence followed the excision of the primary tumor.

The tumor in the first case presents an irregularly growing carcinoma originating in an adenoma of the mammary gland (Carcinoma simplex alveolare). The tumor nodule is sharply defined and there is no evidence of transition from normal gland to adenoma. Transitions between the glandular and the irregularly-growing carcinomatous tissue are, however, apparent in certain portions.

An adeno-carcinoma which developed on the neck of a Japanese waltzing mouse is of a peculiar type. Many of the epithelial cells contain fat, and there are nests of large vacuolated cells, so that the structure of the sebaceous follicles and preputial glands is closely simulated. In portions of the tumor the epithelium is intimately intermingled with the connective tissue. Here the epithelial cells are compressed and spindle-shaped.

The third subcutaneous adeno-carcinoma presents everywhere irregular narrow fissures in the epithelium (" Spaltenbildendes carcinom "). The unequal growth of the glandular epithelium has resulted in a tumor irregularly papillary in type. The epithelium is found growing in the lymphatics.

The fourth subcutaneous adeno-carcinoma, which recurred after excision, presents various types of epithelial growth and shows a marked tendency to hemorrhage (Cystocarcinoma hemorrhagicum). The glandular portions of this tumor contain colloid material.

5. Six of this series of spontaneous tumors have been inoculated into normal mice. Of four subcutaneous adenocarcinomata, two grew on transplantation, one failed to grow, possibly by reason of an accompanying infection, and with the fourth the results of the transplantation were negative. The inoculation of a rapidly-growing lympho-sarcoma into normal mice resulted negatively. Inoculations with a papillary cyst-adenoma of the lung furnished only negative results.

6. Spontaneous tumors originating subcutaneously appear to metastasize much more frequently than either Jensen's or Ehrlich's "Stamm 11" inoculable tumors similarly situated. The lymph nodes are frequently invaded by the spontaneous tumors, but this has never occurred with the above inoculable tumors, in which metastasis always takes place by way of the blood vessels.

7. An inoculable tumor in a Japanese mouse differs from the Jensen and Ehrlich "Stamm 11" tumors in that it grows into the lymphatics.

8. The papillary cyst-adenomata of the lung and the lympho-sarcomata were found in both males and females. The other types of tumors were found only in females.

9. No special significance is found in the pathological processes accompanying tumors in mice. In many instances such conditions as result from infections with a variety of microörganisms, or possibly from old age, are regarded as coincident with but not immediately related to the development of the tumors.

10. The occurrence of primary tumors in mice previously inoculated is not sufficiently frequent to be regarded other than as instances of coincidence.

11. Double primary tumors are of frequent occurrence.

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DESCRIPTION OF PLATES.*

Plate VII.

Fig. 1. — A minute primary tumor of the lung in a mouse, Case No. 9. There is a large blood vessel extending through the central portion of the tumor.

Fig. 2. — A papillary cyst-adenoma of the lung, Case No. 1. Lung tissue is present at the sides of the tumor mass. The surface of the tumor is slightly depressed over its central portion where it has been replaced by connective tissue.

Fig. 3. — A small papillary tumor of the lung, Case No. 3. At the lower right hand border of the nodule is a collection of cells of a type distinct from those of which the tumor is composed. They are probably desquamated alveolar epithelial cells.

Plate VIII.

Fig. 4. — A higher magnification of the tumor represented in Fig. 2, showing the epithelium covering the folds and processes of connective tissue.

Fig. 5. — The periphery of the same tumor, Case No. 1. The tumor is apparent in the right hand portion of the photograph. Individual tumor epithelial cells are to be seen here and there on the alveolar walls. The alveoli are filled with cells of a type distinct from the tumor epithelium.

Fig. 6. — A primary cyst-adenoma of the lung, Case No. 2, stained with Weigert's elastic tissue stain. There is an increase in the amount of elastic tissue in that portion of the pleura covering the tumor. The tumor contains a larger amount of this element than does the normal lung tissue.

Fig. 7. — A hyaline body situated within a nucleus of an epithelial cell of a tumor of the lung. These are frequent in tumors of this type in mice, but occur in other tissues as well.

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FIG. 8.—A papillary cyst-adenoma of the lung, Case No. 8. The tumor has grown into a small bronchus where it is distinctly papillary in type.

PLATE IX.

FIG. 9.—A cyst-adenoma of the kidney, Case No. 10. The nodule consists of a mass of epithelial tissue invaginating a cyst. The nodule is distinct from the kidney tissue below.

FIG. 10.—A cyst of the kidney with irregular proliferation of its lining epithelium, showing the possible mode of origin of certain forms of cyst-adenomata in the kidney.

FIG. 11.—Cyst adenoma of the kidney, occurring in Case No. 1. The kidney tissue occupies the lower left hand portion and the adenoma the upper right hand portion of the field.

FIG 12.—Cysts occupying the wall of the uterus, Case No. 14, in which there is also a subcutaneous adeno-carcinoma.

PLATE X.

FIG. 13.—A subcutaneous adeno-carcinoma, occurring in an old female mouse, Case No. 13, inoculated several weeks previous with the Jensen tumor. Near the border of the tumor a portion of the mammary gland is apparent. The peripheral portion of the growth is purely glandular, but in its central portion the arrangement of the epithelium is very irregular and there is evidence of active growth.

FIG. 14.—A lympho-sarcoma which appeared in the inguinal region of an old female mouse, Case No. 11. The tissue is of the type found in the germinative centers of lymph follicles. The cell outlines are hardly distinguishable. x 1,000 diameters.

FIG. 15.—A portion of the subcutaneous adeno-carcinoma of a Japanese waltzing mouse, Case No. 14. Within the lobules groups of cells become fatty and break down to form spaces so that structures are formed similar to the sebaceous follicles.

FIG. 16.—A portion of the metastasis of the adeno-carcinoma of Case No. 13, shown in Fig. 13. Near the center are structures having somewhat the character of epithelial pearls.

PLATE XI.

FIG. 17.—A subcutaneous adeno-carcinoma in a Japanese waltzing mouse, Case No. 14. The tumor is peculiar in that groups of cells contain fat so that structures occur which resemble very closely the sebaceous follicles of the skin. The epithelium and connective tissue are intimately intermingled, and the epithelial cells are often spindle-shaped. The stroma of the tumor is quite cellular and there are many compound granule and plasma cells.

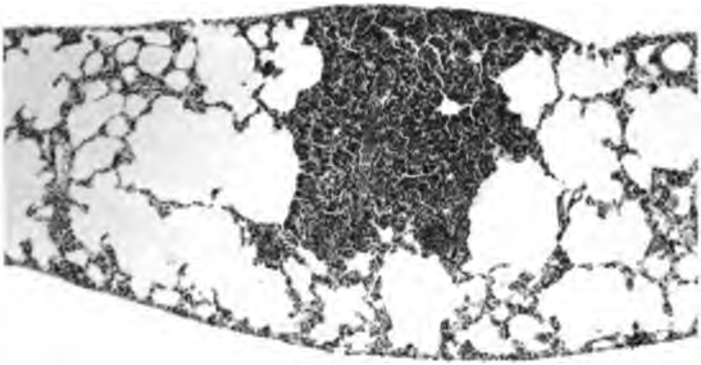
FIG. 18.—A tumor nodule interpreted as a metastasis of the tumor shown in the figure preceding. This was found in the inguinal region,

whereas the larger tumor was found in the neck. The structure of the preputial gland of the mouse is clearly simulated in this smaller nodule.

PLATE XII.

FIG. 19. — A papillary adeno-carcinoma of subcutaneous origin, Case No. 15. The primary tumor as well as the metastasis shows a constant structural peculiarity in the formation of irregular clefts in the epithelium.

FIGS. 20, 21, 22. — Cysto-carcinoma hemorrhagicum, Case No. 16. Photographs taken from different portions of the tumor to show different types of structure. In the first figure the vascularity of the tumor combined with the tendency of its epithelium to form spaces is indicated. The portion of tumor next shown is glandular in structure, and contains colloid material. In the last figure there appears to be great proliferation in the connective tissue, which is invading the tumor.



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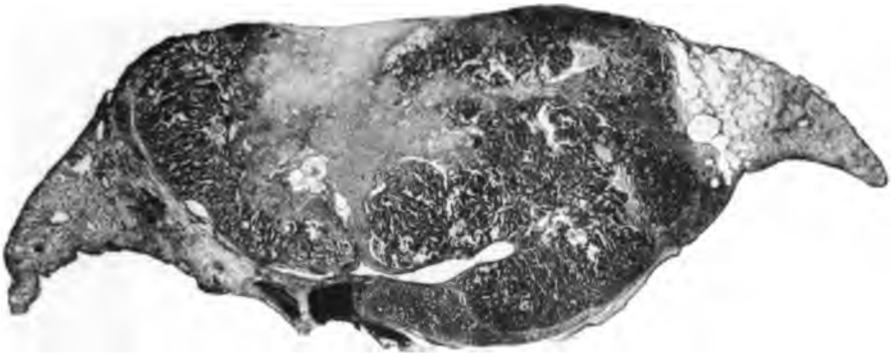

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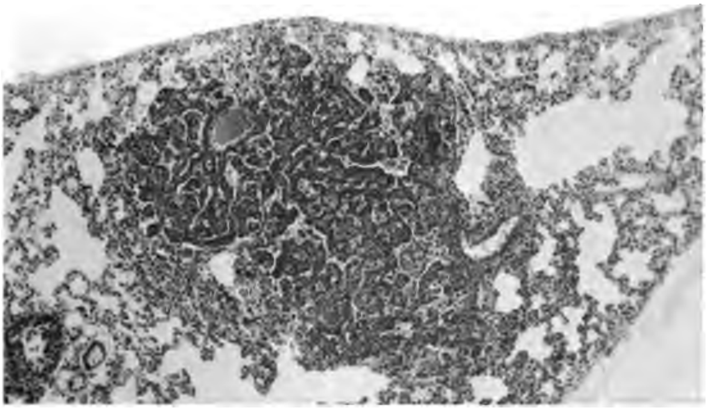

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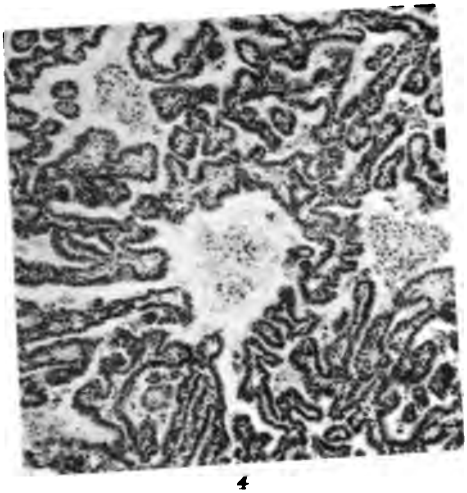

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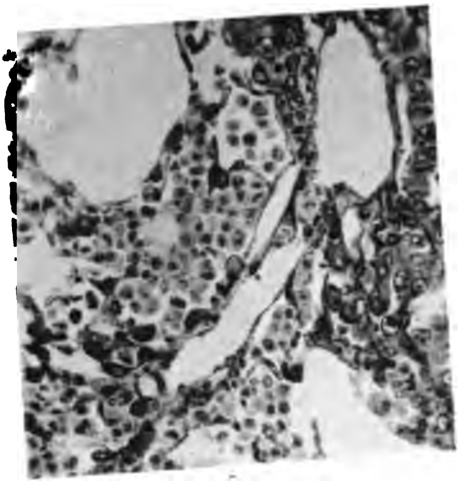

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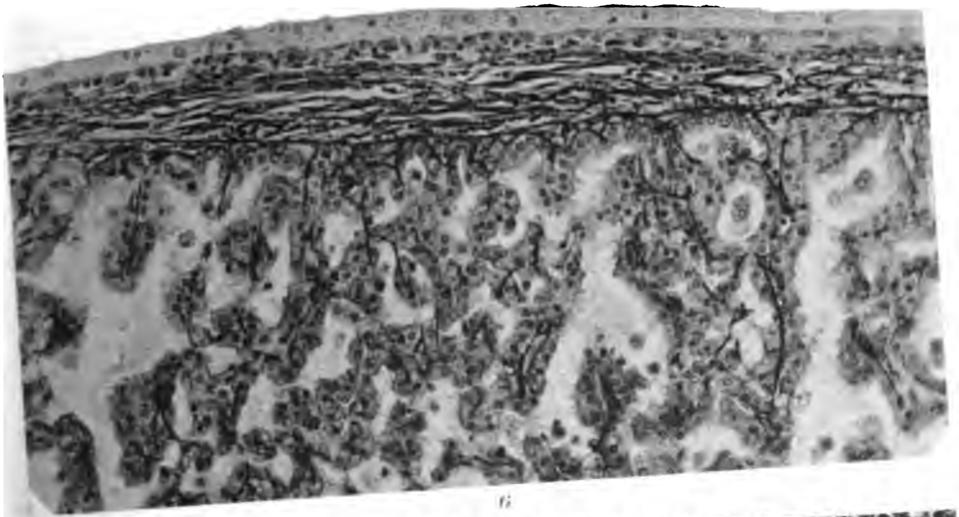

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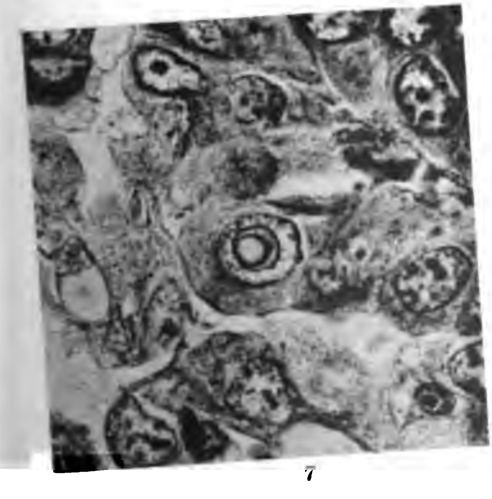

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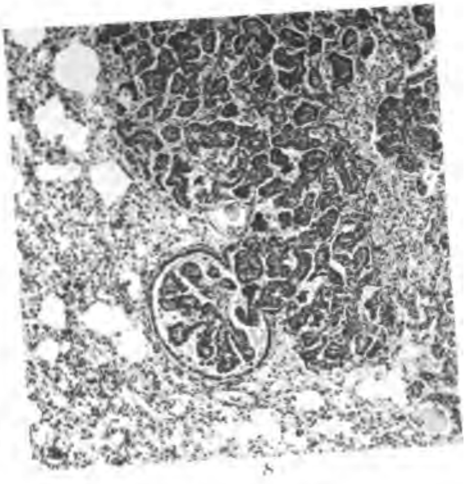

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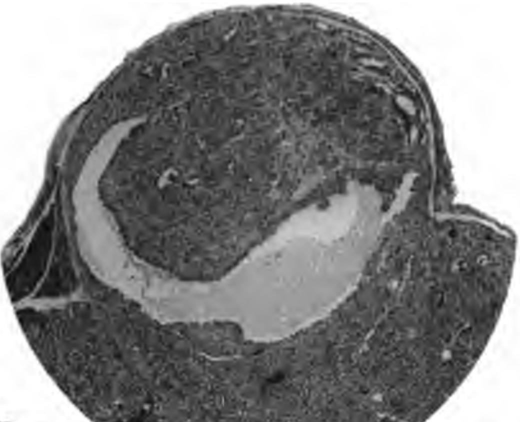
Spontaneous tumors.

Tyzzer.



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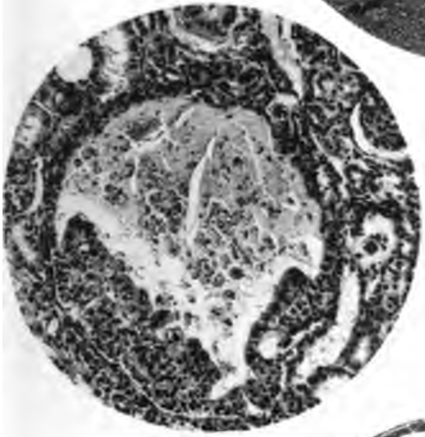

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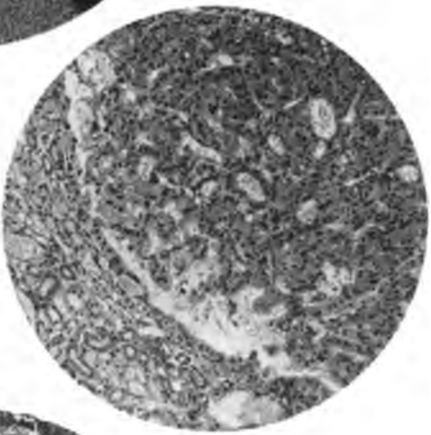

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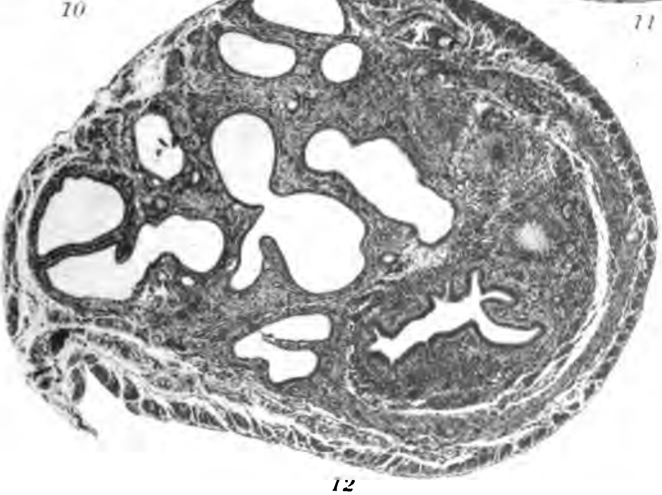

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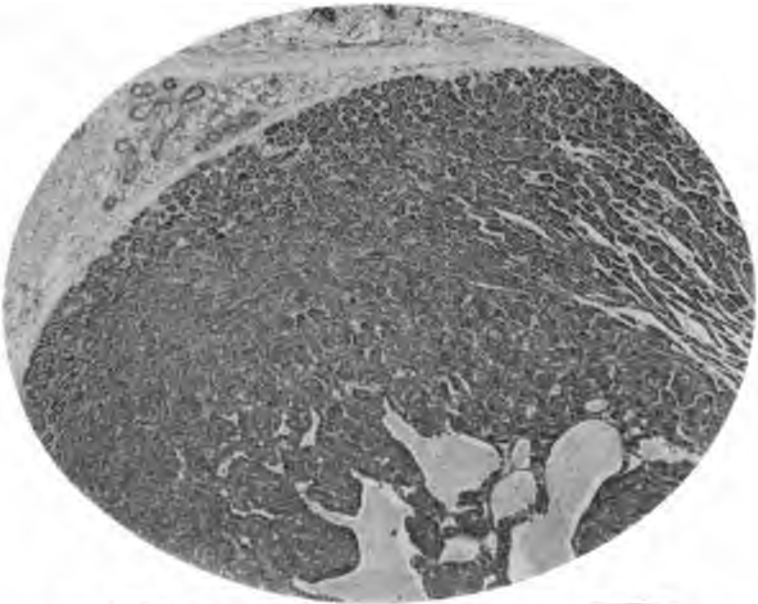

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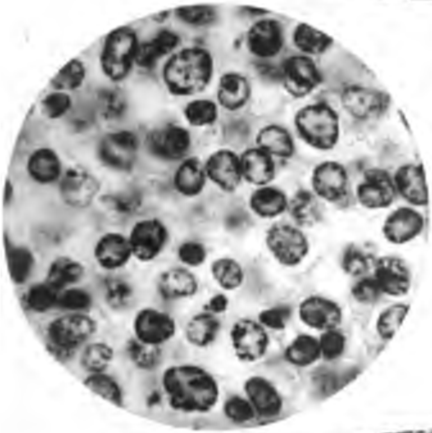

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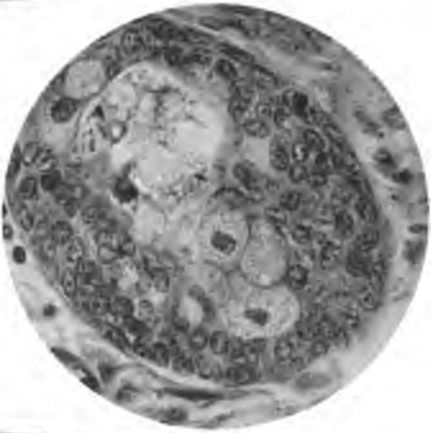

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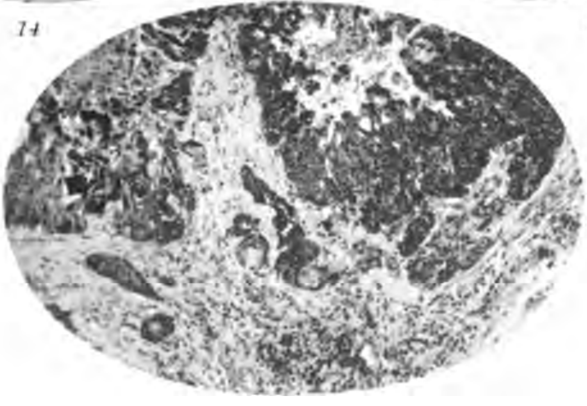

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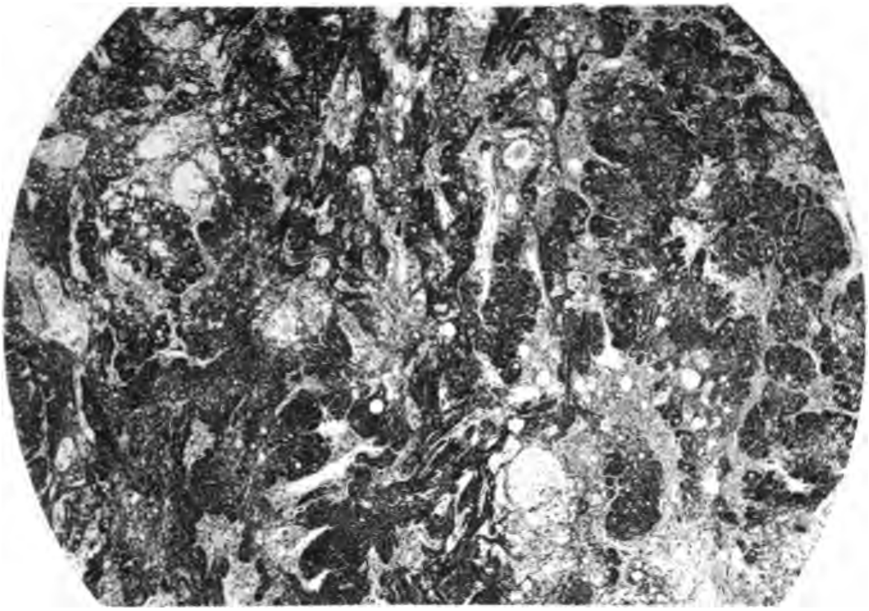
Tyzzer. Spontaneous tumors.



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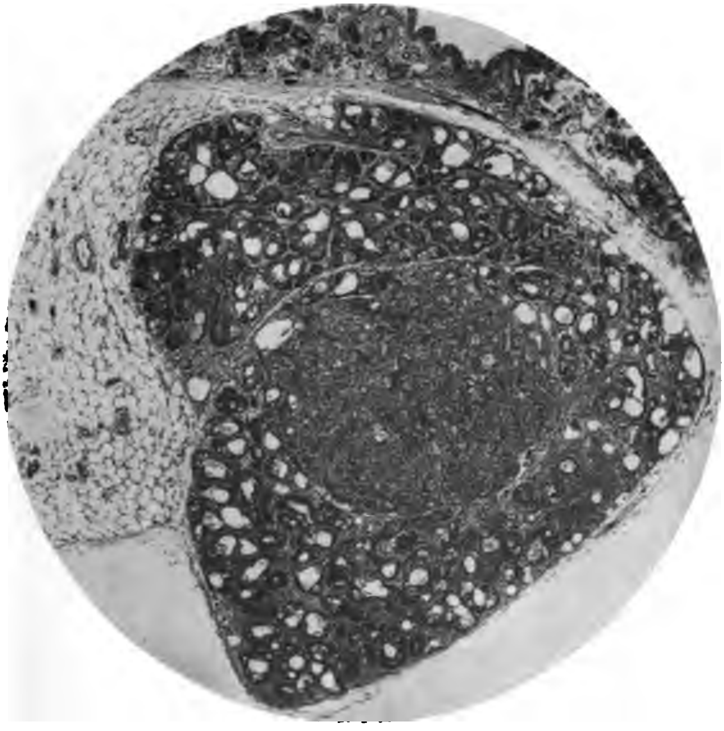

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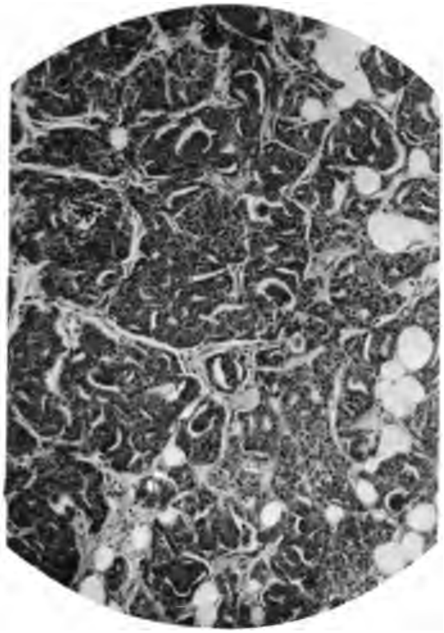

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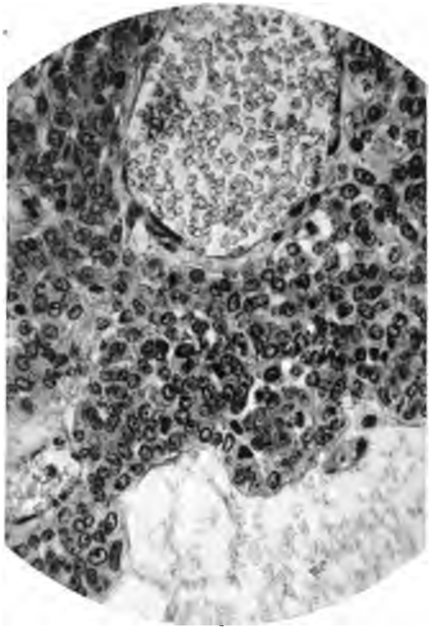

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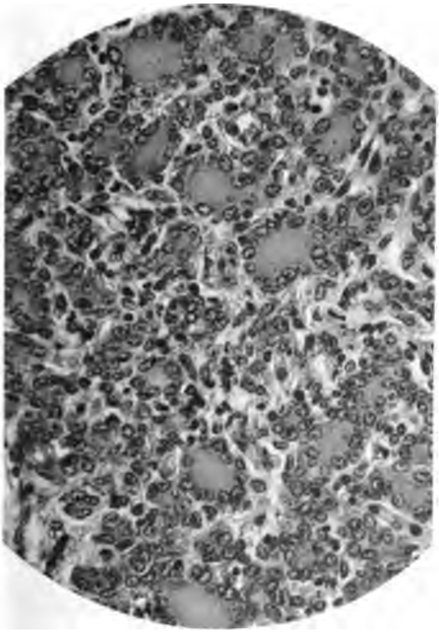

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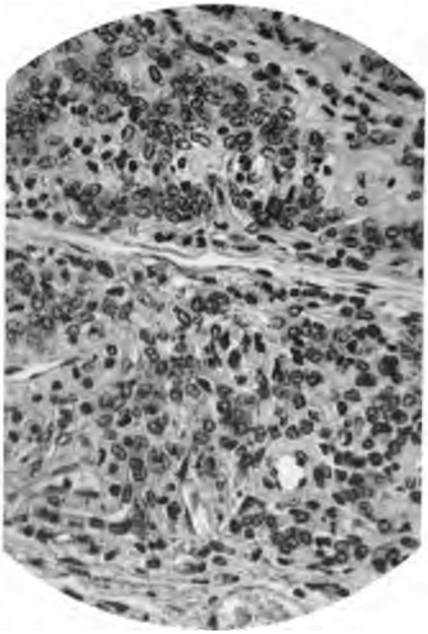

22

A STUDY OF HEREDITY IN RELATION TO THE DEVELOPMENT OF TUMORS IN MICE.*

E. E. Tyzzer.

The statistical methods which heretofore have been applied in the study of the occurrence of cancers in human beings have not as yet furnished proof that the development of these tumors is due to a specific inherited character. In certain families,[1, 2] however, malignant tumors have been so frequent as to attract attention, and the literature bearing upon this subject is considerable. A notable example is found in the family of Napoleon Bonaparte; his father, one of his brothers, and two of his sisters succumbed to cancer of the stomach, as did he himself. On the other hand, the analysis of data derived from a large number of human cases has failed to furnish evidence supporting the view that a predisposition to cancer is inherited.[3] It seems important that the investigation of this question should be carried further, and that use should be made of the experimental method. Since mice breed so rapidly and so frequently develop tumors, they are especially adapted to experimentation of this sort. By regulating the breeding of these animals it is possible to obtain a large amount of accurate data.

The experimental inoculation of different races of the common mouse (Mus musculus) with certain of the inoculable tumors of this species demonstrates beyond question that the tissues of some races are favorable to the growth of these tumors, while the tissues of other races of mice are not. The question arises, — are the races of mice which are susceptible to the inoculable tumors more liable to spontaneous tumors than are the races which are insusceptible? Since heredity is so important a factor in the development of the inoculable tumors, its influence should not be disregarded in the occurrence of spontaneous tumors. If, by selection, a

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race of animals could be obtained nearly all of which developed tumors, even as animals are bred for color or for long hair or short hair, the inheritance of tumors would be established.

There are, however, many difficulties which arise in the way of any such procedure in the breeding of mice. The presence of an internal tumor cannot usually be determined until the death of the animal. Since it is practically impossible to keep all the progeny of all mice, it often happens that the individuals in which tumors are found on post-mortem examination have no available offspring from which to carry on experimental breeding. Mice, when they have developed distinguishable tumors, are usually incapable of further reproduction. In instances in which it is possible to remove an externally situated, primary tumor by operation, the animal is greatly weakened by the procedure, or metastasis has already taken place. On the other hand it would be only by extreme good fortune that offspring could be obtained of parents both of which possessed tumors. This will at once appear evident when it is considered that the adeno-carcinoma of the mammary gland, the most common of the tumors which develop externally in mice, rarely occurs in the males. Furthermore, the mere rearing of a large number of the offspring from mice with tumors can of itself furnish no definite results. Age must be taken into account and all individuals made to live as long as possible.

If the tendency to develop neoplasms be considered an inherited character, the greatest difficulty of all arises in its determination. It is well known that characters exist where they are not distinguishable; thus the color character in certain mammals is present in the embryo, even in the fertilized ovum, although it may be impossible to determine it until later under the conditions of extra-uterine life. Likewise it would not appear improbable that there might be a "tumor character" in certain individuals in which it was not in evidence, but which would possibly become apparent under certain conditions such as those of the menopause or of advanced life.

There is evidence to show that various influences are concerned in the atypical growth of animal tissues. The excitants may be primarily chemical, or mechanical, or of the nature of certain forms of rays and emanations. Concerning the latter, the frequency of carcinoma on the hands of Roentgen-ray operators is well known. Also in certain cases of melanoderma, which closely approaches a neoplasm in its nature, the condition is greatly influenced by light, and the exposed portions of the skin are most affected. While the part played by such external influences is not to be discredited, it is necessary to consider also the possibility that certain individuals possess a tumor character which, under usual or what might be termed "normal" influences, such as are exemplified by light rays in case of melanoderma, may become apparent in the development of a neoplasm. That is, in the origin of the tumor the inherited character constitutes the specific factor.

Whether the tendency to the frequent development of tumors is based on one general or on various specific tumor characters is a question to be considered. In the one case the tumor character will be revealed in the development of tumors of various types; in the other, in the development of tumors of a single type.

The occurrence of cell "rests" or misplaced tissue may possibly have some relation to the inherited tumor character.

If, on the hypothesis that the tendency to the development of tumors is inherited, use is made of the principles of Mendel's law, it becomes at once apparent that the tumor character cannot be a dominant one. If the tumor character should be dominant, then the breeding of an insusceptible individual with one having a tumor would result in hybrids in all of which the tumor character would be dominant. Such a condition as this would be so obvious that it could not fail to have been recognized long before now. If the tumor character is recessive, then the hybrids resulting from the union of an insusceptible individual with one with a tumor character will not in any case show tumors. When, however, these hybrids are bred *inter se*, we should expect twenty-five per cent of the individuals to present the recessive or tumor character. Thus, if (a) stands for the tumor character and (A) for the "no tumor" character, then according to the well-known formula the breeding of two individuals, one with character (A), the other with character (a), results in hybrids Aa, Aa, Aa, Aa, etc., the union of any of which furnishes AA, 2Aa, aa. Thus it is seen that, if the tumor character exists as such, it will most probably be recessive rather than dominant.

It is also possible that the development of a tumor depends upon a combination of several inherited characters rather than upon the presence of a single character. If such is the case it may prove a difficult matter to discover any influence of heredity in the growth of tumors.

That the color character has no relation to the development of tumors is evident from the fact that tumors have been found in white, brown, black, gray, and pied mice.

The following breeding experiments were begun in August, 1905, when a mouse was found with a large tumor of the lung.* The object was primarily to determine whether certain families, as shown by the offspring of mice with tumors, were more liable to develop spontaneous tumors than others. Tumors were subsequently found in other mice, and their offspring were also used for breeding purposes. The method followed has been to keep a record for each mouse, noting the date of birth, description, sex, color, and any apparent disease or change in the general condition which occurs at any time during the entire life. The mice are kept under good hygienic conditions, and are made to live as long as possible. They are killed only when death appears imminent.

Three families of considerable size have been derived from three mice in which tumors developed spontaneously. All three tumors were in races susceptible to the inoculable tumors. As a control a race of mice was taken in which the inoculable tumors had always failed to develop.

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* A similar line of investigation has been suggested by Bashford. The Lancet, 1906, CLXXI., 305.

Since in two families the observations are not as yet ended, the data thus far collected are not sufficient to allow of any conclusions. It is only with the third family that the observations are complete.

Family A. This consists of the offspring resulting from the union of a black female mouse * (Harvard origin) which developed a large papillary cyst-adenoma of the lung, with a white male of a race (Providence origin) insusceptible to the Jensen tumor. The female was black and the male white, so that inheritance as regards the color character could readily be followed in the offspring. Of the young of this pair certain ones were black and others white, so that the female must be considered a hybrid as regards color, for if she had been pure black all the young would have been black, since black is dominant over white. Two young were reared by the female just before the tumor caused her death, and it is from these two that the entire family has been derived.

In certain instances mice of the same generation were bred together, in others mice of different generations.

After the family numbered one hundred and one, breeding was stopped and the mice are now kept under observation. Thirty-six of the total number have died. In none of these were there any tumors found. Four of this number, three males and one female, were over six months old, the oldest being about eleven months at the time of its death, but most of the others were young mice which died before they became fully mature. Six mice were partially eaten by others, so that it was impossible to determine whether there was any internal tumor present.

Of the living mice of this family, one male has attained the age of two years, another male and female one year and six months, and a male one year. All the others are less than a year old, and the majority are not over seven months.

Although up to the present time no tumor has been found in this family, the age thus far attained by any of its members is not sufficiently great to make these results of

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* Case No. 1 of Series of Spontaneous Tumors.

value. The fact that this family is derived from crossing the female presenting a tumor of the lung with a male of a breed insusceptible to the inoculable tumors must be considered. About forty per cent of the offspring are black, thus presenting the color character dominant in the original mongrel female with the lung tumor, but most of these are hybrids, and no more than five are pure black.

A lympho-sarcoma has developed in one of a lot of mice obtained by mating a member of Family A with a mouse of Bashford origin.

Family B. A brown female* (Harvard origin), mated with a white male mouse of another stock (Buffalo origin), reared thirteen offspring. Most of these were used for inoculation and were found susceptible to both the Jensen and the Ehrlich "Stamm 11" tumors. The Jensen tumor in one of these mice† grew very slowly, and, when the mouse was killed, at the age of five and a half months, a primary adeno-cystoma was found in the lung. Of the other twelve mice two escaped, five were ten or more months old when they died, and the remainder died when a few months old. None of these developed primary tumors.

When the male from which this stock originated died, the female was mated with one of the young male offspring. Before the two young offspring resulting from this union were fully mature, the mother developed a lympho-sarcoma (Case No. 11).

The offspring resulting from the union of the female which developed lympho-sarcoma with one of its male young now number eighteen. All but one of these are alive and one year is the maximum age attained.

In this family which has been derived from a female mouse having a lympho-sarcoma, thirty-one offspring have been reared, and twenty-nine have been kept under observation. Six of these died at ages ranging from ten and a half months to fifteen months, but developed no tumors. One of the others, which had been inoculated with the Jensen tumor, died at the age of five and a half months, and presented a primary tumor of the lung. This was six months previous to the development of the lympho-sarcoma in its mother. Of the living members of this family one is a year old, the others considerably younger.

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* Case No. 11 of Series of Spontaneous Tumors.

† Case No. 2 of Series of Spontaneous Tumors.

Family C. The pair of mice from which this family originated were purchased of a dealer in Detroit when quite young and were probably brother and sister of the same litter. Their offspring were found susceptible to the inoculable tumors, and many of them were thus used for inoculation purposes. On the death of the male, at the age of fourteen months, a minute tumor was found in the lung.

The offspring of this pair of mice number twenty-four. All are dead, and a post-mortem examination has been made of each mouse. Tumors of the lung, identical in type with that of the original male, were found in three of the offspring, one in each of the three generations. Thus in the entire family of twenty-six mice there were four tumors of identical character.

Two of the tumors occurred in mice previously inoculated, one with the Ehrlich "Stamm 11" tumor which grew to a large size, the other with a lympho-sarcoma which was never successfully inoculated. The other two tumors which occurred in this family were in mice which had never been inoculated.

The tumors were equally represented in both sexes, two occurring in males and two in females. The entire family consists of thirteen males and thirteen females.

Two of the mice, a male and a female in which the tumors developed, were between eight and nine months of age, the other two, a male and a female, were over a year old. The former two had never been inoculated, the latter two had been inoculated with tumor tissue.

Of the remaining twenty-two negative mice, eleven were never inoculated; two were inoculated with the Jensen tumor which grew in both, eight were inoculated with the Ehrlich "Stamm 11" tumor which grew in three, and one was inoculated with a lympho-sarcoma with a negative result.

The ages attained by the negative mice ranged from one to fourteen months.

The tumors were found on the following dates: May 10, July 10, July 23, October 22, of the same year.

Hybrids of Family C. The results of cross-breeding members of Family C, which represents a pure stock, with other mice are also to be considered. Mice of various other stocks, and in some instances mixed stocks, were used in this cross-breeding. Forty-four of the resulting offspring have been autopsied. All but one of these had been previously inoculated with either the Jensen or the Ehrlich "Stamm 11" tumor. Most of the mice in which the inoculated tumors

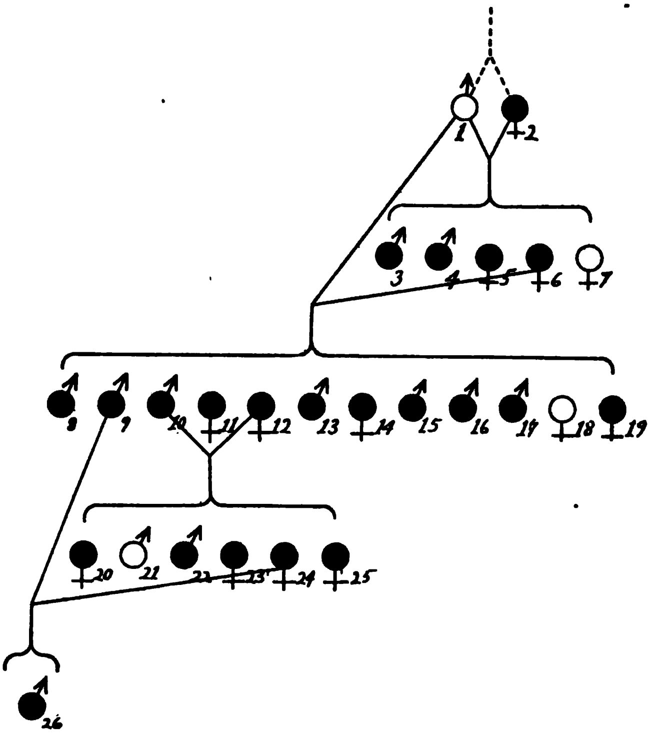

Chart showing the occurrence of tumors in Family C:
○ = mice which developed tumors.
● = mice in which no tumors were found.

grew succumbed at an early age, only two living to six months. Many of the mice in which the inoculated tumors failed to grow lived to a much greater age.

One, a hybrid female, the offspring of a member of Family C (Detroit origin) and a mouse derived from the union of a member of Family A with a mouse of the stock of the ancestral female of that family (Harvard origin), had never been inoculated. It died at the age of eight months and presented a primary papillary cyst-adenoma of the lung* similar to those found in the two families from which it was derived.

Of the forty-three inoculated hybrids which had been derived by mating certain members of Family C with mice of various stocks, two † presented similar tumors of the lung. These were two females of a single litter resulting from mating a male of Family C with a hybrid female, derived from the union of mice of two different stocks (Harvard and Bashford), in both of which tumors of the lung have occurred. Both of these mice had been inoculated with the Jensen tumor. In one a nodule appeared at the site of inoculation, grew for a time, but was later on absorbed. Both of these mice in which the tumor of the lung was found died when slightly less than a year old.

Thus, primary tumors of the lung occurred in only two of the forty-three inoculated hybrids, whereas the only uninoculated hybrid presented a tumor of this type. From these data and from the infrequency of primary tumors in a long series of mice inoculated with various types of tumors, there does not appear to be any relation between such inoculations and the development of primary tumors. In order, however. to obtain more data on this point, it might be well to repeat the procedure followed in Family C, and inoculate one-half the members of other susceptible families so as to determine the frequency of primary tumors in equal numbers of inoculated and uninoculated mice of the same family.

Family D is derived from mice purchased from a

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* Case No. 7 of Series of Spontaneous Tumors.

† Cases Nos. 8 and 9 of Series of Spontaneous Tumors.

Washington dealer. Since the inoculation of these mice with either the Jensen or the Ehrlich "Stamm 11" tumors gave invariably negative results, the family was taken as a control for the hypothetically susceptible families A, B, and C. This family has been kept under observation for over two years and a careful post-mortem examination has been made of all mice that have died. No tumor has been found in any case. Of twenty-three that have died, two were more than a year old and all the others were adults of unknown age. The members of this family are kept together in two large cages, which are seldom cleaned and then not very thoroughly, so that the ideal conditions are furnished for cage infection.

In working up the data of these breeding experiments a mistake has been found in that the experiments were made too complicated. Thus where individuals of different generations have been bred together, the experiment becomes too involved for the interpretation of results.

The fact that the character in question is, in many cases, not apparent adds to the difficulty. These experiments are thus to be considered as being somewhat preliminary in nature, but indicate, nevertheless, a line of work which is certain to lead to definite results whether positive or negative; that is, it seems possible by experimental procedure to determine whether there is a tumor character inherited in accordance with Mendel's law.

It has already been pointed out that if such a character exists it is probably recessive. In this case if two animals with the tumor character are mated together all the offspring should possess the tumor character. This is possibly the case in Family C, but it is then necessary to assume that the tumor character, present in all the mice of this family, became apparent only in four individuals.

According to Mendelian principles, if one of the parents possess the recessive tumor character and the other the dominant "no tumor" character, the first generation of offspring would be hybrids in all of which there would be no evidence of the recessive tumor character. However, by breeding these hybrids together, twenty-five per cent of the young would possess only the "no tumor" character, fifty per cent would be hybrids with the "no tumor" character dominant, and twenty-five per cent would possess only the recessive tumor character. On this basis it will not be difficult to compare, as regards the development of tumors, the second generation of the offspring of an insusceptible male and a tumor female with the offspring obtained by breeding the same male with an insusceptible female. A breeding experiment of this sort has just been commenced.

The results of the previous breeding experiments may be briefly summarized as follows:

Family A, which has been derived from a female mouse, with a papillary cyst-adenoma of the lung, mated with a male of a stock insusceptible to the inoculable tumors, has furnished no offspring presenting tumors. The observations are, however, not yet complete since the majority of the mice are still alive and many are still young.

Family B originated by mating a female which developed a lympho-sarcoma with a male of a stock susceptible to the inoculable tumors. This also furnishes rather meager data, since the mice now living are relatively young, and most of those already dead succumbed at an early age. One of the offspring, however, which had been inoculated successfully with the Jensen tumor, presented also a papillary cyst-adenoma of the lung at the age of four and a half months. In this instance the primary tumor in the offspring was of a type distinct from that of the tumor of the parent.

Family C originated with a pair of mice, evidently of the same litter, the male of which presented a minute papillary cyst-adenoma of the lung. In this family three of the twenty-four offspring developed tumors of the lung of the same type as that which occurred in the first male.

The cross-breeding of certain members of Family C with mice of other stocks has resulted in offspring, forty-four of which have been autopsied. All but one of this number had been previously inoculated with either the Jensen or the Ehrlich "Stamm 11" tumors. The one uninoculated mouse and two of the forty-three inoculated mice have developed tumors of the lung similar to those found in Family C.

Family D, which consists of a race of mice insusceptible to the inoculated tumors, has not yet furnished an example of a spontaneous tumor.

All these tumors occurred in mice which were not extremely old, so that their frequency is the more important.

That the frequency of occurrence of the papillary cyst-adenoma of the lung is the same in the noninoculated as in the inoculated mice is indicated in Family C, in which an equal number of each at about the same age may be compared.

It seems possible that heredity may explain the occurrence of tumors among mice kept in certain cages, instead of cage infection, as has been the belief of several investigators. If infection or other conditions have some remote causal relation to the development of tumors, then the relatively great susceptibility of certain families and the insusceptibility of others to spontaneous tumors is indicated thus far in the breeding experiments. This is especially applicable to the occurrence of the papillary cyst-adenoma of the lung. Although the conditions furnished the family of control mice were most favorable for infection, yet no tumor has developed among them.

Families which show greatest insusceptibility to the inoculated tumors are less liable to spontaneous tumors, although this may possibly not always hold true for individuals. As regards the latter question, however, it is difficult to determine, in a mouse in which a primary tumor is found after an inoculated tumor has failed to grow, whether the primary tumor was already in existence before the inoculation or developed subsequently. Although in certain cases the lung tumors were found many months after the failure of the inoculable tumor to grow, they may possibly have been so slow of growth as to have existed at the time of the inoculation. On the other hand, the failure of the inoculable tumor to grow does not invariably indicate the insusceptibility of the animal, for in a certain number of cases second inoculations have been successful.

In conclusion it may be stated that the results of these breeding experiments are not thus far sufficiently numerous to prove or disprove that the development of a tumor is dependent upon the presence of an inherited character, although the data obtained may appear to favor this view. It seems possible by certain lines of experimental breeding, with the principles of Mendel's law of heredity in mind, to obtain definite results, whether they be positive or negative.

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THE FIFTH REPORT

OF

THE CANCER COMMISSION OF HARVARD UNIVERSITY

(Founded by Caroline Brewer Croft, June 16, 1899)

(FORMERLY THE CAROLINE BREWER CROFT FUND CANCER COMMISSION)

The Medical School of Harvard University

BOSTON
MASSACHUSETTS
1909

THE FIFTH REPORT

OF

THE CANCER COMMISSION OF HARVARD UNIVERSITY

(FOUNDED BY CAROLINE BREWER CROFT, JUNE 16, 1899)

NOVEMBER 1, 1909.

DEAR SIR:

The Fifth Report of The Cancer Commission of Harvard University (formerly The Caroline Brewer Croft Fund Cancer Commission) is herewith submitted for your consideration. Should you desire to receive the future reports of this Commission as they may be issued, will you kindly acknowledge the receipt of this report and send your full address to the Secretary at your earliest convenience.

Very truly yours,

J. COLLINS WARREN, *Chairman.*

ROBERT B. GREENOUGH, *Secretary*,
Medical School of Harvard University,
Boston, *Mass.*

◆

THE MEDICAL SCHOOL OF HARVARD UNIVERSITY
BOSTON, MASSACHUSETTS

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CONTENTS.

PAGE

THE LESIONS OF THE SKIN AND THE TUMOR FORMATIONS IN XERODERMA PIGMENTOSUM.
W. T. Councilman and G. B. Magrath 5

THE SURGICAL TREATMENT OF X-RAY CARCINOMA AND OTHER SEVERE X-RAY LESIONS, BASED UPON AN ANALYSIS OF FORTY-SEVEN CASES.
C. A. Porter 31

THE PATHOLOGICAL HISTOLOGY OF CHRONIC X-RAY DERMATITIS AND EARLY X-RAY CARCINOMA.
S. B. Wolbach 89

CHRONIC PANCREATITIS WITH TUMOR-LIKE NODULES IN THE CAT.
Thomas Ordway 125

TUMORS IN THE COMMON FOWL.
E. E. Tyzzer and Thomas Ordway 133

A SERIES OF SPONTANEOUS TUMORS IN MICE WITH OBSERVATIONS ON THE INFLUENCE OF HEREDITY ON THE FREQUENCY OF THEIR OCCURRENCE.
E. E. Tyzzer 153

A STUDY OF INHERITANCE IN MICE WITH REFERENCE TO THEIR SUSCEPTIBILITY TO TRANSPLANTABLE TUMORS.
E. E. Tyzzer 193

THE NATURE OF THE REACTION OF THE TISSUES OF SUSCEPTIBLE AND NON-SUSCEPTIBLE MICE TO AN INOCULABLE TUMOR.
A. M. Burgess 249

THE EFFECT OF TRYPSIN ON CANCER AND ON THE GERM CELLS IN MICE.
Stephen Rushmore 265

A TRANSMISSIBLE CANCER OF THE RAT CONSIDERED FROM THE STANDPOINT OF IMMUNITY.
F. P. Gay 271

THE FIFTH REPORT

OF

THE CANCER COMMISSION OF HARVARD UNIVERSITY.

THE LESIONS OF THE SKIN AND THE TUMOR FORMATIONS IN XERODERMA PIGMENTOSUM.*

W. T. COUNCILMAN AND G. B. MAGRATH.

The purpose of this paper is to record the results of the examination of the tissues in two fatal cases of xeroderma pigmentosum. This disease was first described as an entity by Kaposi in 1882, and few diseases can be regarded as more typical in the inception, course, and termination. In the literature the histories and descriptions of cases with minor and unimportant differences are repeated.

The disease usually begins before the second year in children who have previously been healthy. The first change noted is an erythema with more or less inflammatory edema following exposure to sunlight, which persists longer than usual and is repeated on similar exposures. The erythema is followed by pigmentation which occurs in larger spots and is usually darker than the ordinary freckles. In some cases the pigmentation was the first change noted. The pigmentation is usually in circumscribed patches but may be diffuse, and both the dark freckles and lighter diffuse areas of pigmentation may be present together. The pigmentation increases and at the third or fourth year the skin, at first smooth, becomes roughened and scaly. Atrophy of the skin then begins and roughening and scaliness increases. In the areas of atrophy the pigment disappears, but it is increased around them. The atrophy affects both the epidermis and corium,

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* Received for publication July 26, 1909.

but the corium is not adherent to the underlying tissue. Hyperemia of the conjunctiva and photophobia develop. The roughening and scaling of the epidermis continue and hard warty elevations appear which represent accentuations of the general keratosis. In the atrophic areas of the skin large dilated tortuous veins, often arachnoid in their arrangement, may be found. The height of the process is reached in the sixth or seventh year. The warty structures become more marked and with them appear tumors of a carcinomatous character. The disease is usually fatal before the twentieth year, although Falceo reports three cases in women aged respectively 72, 89, and 90. This is the only report of the disease at this advanced age and it is not improbable that the condition in Falceo's cases was merely that of senile atrophy of the skin with keratosis, which xeroderma so much resembles that one of the many names proposed as a substitute for xeroderma pigmentosum is senilitas precox. Most of the tumors have been found to be pure epidermoid carcinomas, but in one of Pick's cases a melanotic tumor appeared on the leg, which was examined by Chiari and from the description must be regarded as a melanotic sarcoma, and in another a tumor appeared on the alveolar process which was not examined histologically. These tumors in Pick's cases form such exceptions to the general rule that they should probably be regarded as coincidences and not related to the disease. The mucous membranes are intact.

The lesions are confined to certain parts of the skin affecting those areas which are least protected by clothing. They are more marked and are usually first noticed on the face and neck. The disease affects the hands, extending up the arms and becoming progressively less. The lower extremities are less affected except in the case of children who have been accustomed to go barefoot. The lesions may extend in the lower extremities to the thighs. The tumors may appear on any of the affected areas, but are more common on the places which are usually the site of similar tumors, as around the mouth, the eyelids, and the nose. Neither the skin lesions nor the tumors are accompanied by swelling of the lymph nodes, and metastases do not take place as a rule. In one of Kaposi's cases there was a metastasis in one of the cervical glands, and in another (a woman of twenty-five) death was ascribed to cancer of the peritoneum. In neither of these cases were the supposed metastastic tumors examined histologically.

The disease has a peculiar extension in the family. Two or more cases usually appear in the same family. The largest number in a single family was reported by Ruder. In this one seven out of eight boys were affected, the unaffected boy having died early of croup. Five girls in the same family were free from the disease. The sexes are about equally affected, but when the disease appears in a family it is limited to either the males or females. The only exception to this rule occurs in Kaposi's cases in which a brother and sister were affected.

Most of the reported cases have occurred in Germany and Austria, but a number have been published in France, Italy, England, and the United States. One case has been reported in Syria, and in this the lesions were particularly marked in the skin left uncovered by the V-shaped opening in the dress below the throat. This seems to be the only case reported of its occurrence in the tropics. There is no evidence of its occurrence in any of the pigmented races. There is no relation to race. Whether more frequent in cities or in country districts cannot be ascertained, as this is not always mentioned and the number of cases is too small to furnish statistics of any value. The etiology is obscure. The opinion most frequently expressed is that there is some congenital defect in the skin which diminishes its resistance to the light rays, especially the ultraviolet rays, and that the lesions are due to light. The pigmentation is regarded as a conservative process designed to afford protection to the skin from the action of the light.

The following two cases, sisters in one family, were observed in the Long Island Hospital. It is to be regretted that the histories are so incomplete.

Case 1, No. 616. — *Clinical history:* E. F., age 8 years. Entered L. I. H. Aug. 23, 1898. Healthy as an infant. At two years of age freckles appeared upon face, neck, and arms, which gradually spread and became larger.

Nov. 15, 1899. Extensive horny growths upon face. Ulceration has partially eroded nose. Vision somewhat impaired. Patient up and about ward. Appetite fair.

July 10, 1900. Patient has been in cottage built for her use for about two months. She can distinguish light from darkness, but is otherwise blind. Mass on right cheek has steadily increased in size and is foul from putrefaction. Without anesthesia portions of the mass were trimmed away.

Aug. 21, 1900. Blood examination (Dr. E. A. Locke): reds 1,350,000; leucocytes 25,800; hemoglobin less than 10 per cent. Differential count (1,000 corpuscles): small lymphocytes 7, large lymphocytes 1.4, transitional .6; polymorphonuclears 90; eosinophiles .4; myelocytes .6; marked poikilocytosis, with many large erythrocytes, some with pale centers, and many with protoplasm staining uniformly; a few polychromatophilic cells; normoblasts 12.

Aug. 28, 1900. Mass upon face is as large as formerly. Portions of this were trimmed off as formerly, and consequent hemorrhage controlled with pressure.

Aug. 29, 1900. Patient very weak and anemic. Died at about 12.45 P.M.

Autopsy, Long Island Hospital, Aug. 30, 1900. Sixteen hours post-mortem. *Clinical diagnosis:* Xeroderma pigmentosum; anemia. *Anatomical diagnosis:* Macular pigmentation of the skin; atrophy of the skin; multiple nodular tumors of the skin of the face, neck, and arms, with ulceration; pannus; ectropion; fatty degeneration of the heart; fatty degeneration of the liver; cloudy swelling of the kidneys; fatty degeneration of the kidneys; parietal thrombus of the inferior vena cava, with puriform softening; red bone marrow; anemia.

Body: That of a girl 10 years of age; length 123 centimeters; well developed, fairly well nourished; rigor mortis absent; no post-mortem discoloration; marked pallor. Hair bright red.

Head: The skin of the scalp, face, and neck so far as the clavicle is mottled with irregularly outlined, light to dark brown spots, some of them black, from 2 to 5 millimeters in diameter, the smallest resembling freckles, most numerous in the cervical region. The spots are not elevated. In places, the skin is free from pigmentation, and presents a smooth, glistening surface, white, and with some resemblance to the skin of a scar.

The right eye presents at the inner and lower part of the ball a white nodule 1 centimeter in diameter; the conjunctiva is thickened, and the iris opaque; the lower eyelid is everted and contracted downward.

The left eye presents almost complete obliteration of the conjunctival sac; the iris is opaque and presents a nodule similar to but smaller than that upon the right eye; the lower eyelid is everted and eroded.

The alæ nasi are absent; the position of the right is occupied by an oval (2 x 1 centimeters) ulcer, extending to the upper lip, and covered with a greenish, slimy slough.

The right temporo-malar region presents an irregularly outlined, lobulated, somewhat fungoid mass, extending from the outer margin of the orbit to a point about 2 centimeters in front of the pinna, about 6 centimeters in diameter, and with an elevation of about 3 centimeters at its highest point; at its edges, the mass overlies and its easily separable from the underlying ulcerated skin. The edges of the mass are flesh-colored, its main bulk a dirty, grayish green. The peripheral parts of the growth are of fairly firm consistence, the central part friable, soft, and moist. The mass emits a very foul odor.

The lower part of the right cheek presents an elliptical (2 x 4 centimeters) ulcer, extending to the angle of the mouth, the edges elevated, the base granular and red.

The left cheek, just below the left eyelid, presents a similar ulcer, while another occupies the middle portion of the left side of the face.

The left supra-orbital ridge presents two nodular elevations of about the size of a large pea; the outer one horny and friable, the inner covered with soft, somewhat macerated epithelium.

The mid-frontal region presents a shallow, poorly-defined ulcer about 2 centimeters in diameter. A similar ulcer occupies the left frontal region.

The general effect of these lesions is to give the face an excessively disfigured aspect.

Upper extremities: The left arm (upper) upon its anterior surface presents an irregularly-outlined area 6 x 3 centimeters, its long axis parallel with that of the arm, within which the skin is thin, white, smooth, and somewhat glistening; toward the center of the area the skin is very slightly puckered after the manner of a scar. The lateral surfaces of the arm present a few small, light-brown macules. The left forearm, extensor surface, and the dorsum of the left hand present numerous, larger and smaller, mostly about 3 millimeters in diameter, brown to black macules; in places the skin is without pigmentation and presents a smooth, glazed appearance. The flexor and the extensor surfaces of the forearm present multiple, warty, friable elevations, for the most part 2 x 6 millimeters in height, some of them white and moist, others yellow and brown, and dry. The dorsal surface of the first and the second fingers of the left hand present small, horny, elevations.

The right upper extremity presents in general the appearances shown in the left; macular pigmentation, small areas of white, glazed skin, and a few nodular tumors.

Trunk: The skin of the chest, back, and abdomen is white and soft, notable for nothing save pallor.

Lower extremities: The anterior surface of the thighs and the legs presents a very few, widely-scattered pale-brown macules, of about the size of a pin's head, and resembling the ordinary freckle; these are somewhat more numerous about the knees than elsewhere.

Internal examination: Upon section, subcutaneous fat .4 centimeters in thickness, muscles pale and thin. Vermiform appendix and mesenteric lymph nodes normal.

Thorax: Height of diaphragm fourth interspace on right side, fifth rib on left. *Pleural cavities:* Normal. *Pericardial cavity:* Normal.

Heart: Weight, 155 grams; upon section, auricles contain a moderate amount of not very dark, unclotted blood; myocardium pale brown, upon microscopic examination showing the presence of a very large amount of fat; wall of left ventricle .8, of right .2 centimeter in thickness; mitral valve 6.6, aortic valve 4, pulmonary valve 4 centimeters in circumference; depth of left ventricle 7.5 centimeters; valves and cavities normal; coronary arteries normal. *Lungs:* Voluminous, pale salmon-pink; crepitant throughout; upon section, cut surface on pressure yields a slight amount of frothy fluid. Bronchial lymph nodes grayish black. *Cervical lymph nodes:* Of about the size of white beans upon section, homogeneous, pinkish white.

Abdomen: Peritoneal cavity not remarkable. *Spleen:* Weight, 50 grams; purplish red; upon section, markings fairly distinct; dark red; pulp slightly increased; iodine solution gives a somewhat doubtful reaction for amyloid. *Liver:* Weight, 625 grams; pale yellowish brown; upon section, markings fairly distinct; consistence normal; amyloid reaction uncertain. *Gall bladder:* Normal. *Stomach and intestines:* Normal. *Kidneys:* Weight of both 135 grams; upon section, capsule strip easily from a smooth surface; markings distinct; cut surface somewhat opaque; glomeruli not prominent; color yellowish brown; microscopic examination shows the presence of much fat.

Adrenal glands: Normal. *Bladder and genitalia:* Normal. *Aorta:* Normal. *Vena cava inferior:* Upon section, at a point about 5 centimeters above the confluence of the common iliac arteries, presents attached to its posterior and right lateral walls a soft, reddish mass, about 1 x 3 centimeters, partially covered with post-mortem clot; upon section the mass presents within a puriform appearance; smears from the center of the mass show leucocytes and granular detritus, but no bacteria. *Retroperitoneal lymph nodes:* Normal. *Cranium:* Sinuses normal. *Meninges:* Normal. *Brain:* Weight, 1,085 grams; normal. *Tympanic cavities:* Normal. *Spinal cord:* Normal. *Organs of the neck:* Cervical lymph nodes as above noted; otherwise, not remarkable.

Bacteriological examination: Negative.

Bone marrow of the femur showed marked erythroblastic activity. It contained great numbers of nucleated red blood corpuscles and the cells precedent to these. There was also hyperplasia of the myelocyte series of cells and a considerable accumulation of polynuclear leucocytes. In the sinusoids of the liver there were relatively numerous polynuclear leucocytes, a fair number of myelocytes and nucleated red corpuscles. One of the cervical glands examined showed thin lymphoid follicles and strands; no germinal centers were found; the sinuses were small and filled with endothelial cells; no phagocytosis in these cells. The follicles of the spleen were small; no germinal centers present. In all the follicles, in some more marked than in others, there was an eosin-stained coarse reticulum corresponding to the blood vessels. In some cases the reticulum was hyaline, in others it was composed of agglutinated and partly hemolyzed red blood corpuscles.*

The liver contained a few small necroses with infiltration of polynuclear leucocytes about them in the center of the lobules. Examination of the other internal organs showed no lesions other than slight degrees of degeneration. In this case death was due to the general malnutrition and the anemia.

The cutaneous lesions will be described with those of the case following.

CASE 2, No. 854. — *Clinical history:* F. F., age 9 years. Entered L. I. H. Aug. 7, 1901. When one year old face, neck, and hands became covered with freckles. When six and one-half years of age bunches began to grow upon face. Under treatment at the Massachusetts General Hospital for an indefinite period. X-ray treatment at Massachusetts General Hospital for the past four months with benefit.

At time of entrance left eye obscured from ulceration and edema of the lower lid; right pupil normal. Physical examination of chest and abdomen negative. Face presents many horny growths varying in size from small pea to an English walnut; most of them hard and white, some of the larger soft and ulcerated. The skin about the eyes is more or less ulcerated, the ulcerated surfaces yielding slight sero-purulent discharge. On the upper lip is a growth about the size of half an egg. On the hands and forearms are many pigmented areas, and a few very small horny nodules on the backs of the hands. Patient up and about. General condition fairly good. Nov. 14, 1901: Condition less satisfactory; appetite poor; impaired mentality; ulceration about left eye has increased in size and requires frequent cleansing to remove discharge; nodule on upper lip has become quite foul, but has increased little in size; removed. Dec. 10, 1901: Patient weaker; she complains of pain about ulcerated areas; morose. Jan. 8, 1902: Patient in bed; ulceration spreading and growing deeper; discharge abundant and foul-smelling; nodule on upper lip has grown perceptibly and is now the size of a hickory nut. Feb. 13, 1902: Considerable pain, requiring morphia, gr. one-sixteenth every four hours; occasional small hemorrhage from ulceration; lesions dressed and cleansed daily with weak carbolic solution. Feb. 20, 1902: Morphia increased to gr. one-eighth every four hours; ulceration continues to extend in area and depth slowly; growth on lip now about the size of an English walnut; patient much weaker. March 1, 1902: Large growth on upper lip ligated about pedicle and removed; morphia in doses of gr. one-fourth occasionally; ulceration about left eye has extended laterally in depth until its area covers a surface about 3 x 5 centimeters, the lower border of which has extended into the eye so that the eye-ball can be seen, the surface sloughing; bone visible in several places; lesions have extended so that they now include the entire neck down to the shoulders; on the back of the neck is a nodule like the others, but not sloughing, dry, and scaling, about the size of a hickory nut; on the backs of the hands these areas have increased and are now quite numerous and of considerable size; pigmented areas are present upon the shoulders; skin elsewhere negative. March 20, 1902: Patient weaker; complaining of excruciating pain in spite of opiates; area of ulceration extending; discharge not very profuse, but fetid. April 9, 1902: Condition improved; less pain; better appetite; skin lesions show no change. May 1, 1902: Patient weaker; ulceration discharging abundantly; a few small rapidly-growing tumors have appeared upon the right side of face, showing a marked tendency to break down and become part of the general ulcerated area. June 2, 1902: Increasing weakness; ulcerated areas require constant attention. July 5, 1902: Marked emaciation, skin of arms, hands, face, and neck thickly studded with brown freckles varying in size from that of a pea to a dime. On the backs of the hands are horny projections rising 3–4 millimeters above the surface and 3–6 millimeters in diameter; the surfaces of these are dry and scaling; though removable they are quite firmly situated; one of these on the left side of the neck is 1.5 centimeters in diameter. The right ear presents a small ulcerated area in the tragus, the surface pale, moist, and discharging thin, foul, muco-purulent material; it apparently involves the cartilage. On the chin, including about two-thirds of it, and extending chiefly to the right side, is an ulcerated area reaching about to the margin of the lower lip, and below to the border of the jaw, deep enough to involve the bone and the alveolar process, and exposing the teeth, some of which have fallen out; the border of the ulcer shows no inflammatory reaction; the surface pale and oozing; the right border is somewhat undermined. On the upper lip is the base of a large horny growth recently removed; the surface of this is somewhat reddened and presents clotted blood. On the right ala is a superficial greenish slough which extends to the superior border of the nose. On the forehead is a sloughing area extending from the median line to the left, involving the left side of the nose and left orbit down on left malar bone to the ear, below to a line from the angle of the jaw to the tip of the nose; this has extended in depth, so that while the edge is rather superficial, involving only the soft parts, the central portion has destroyed the bony orbit so that the globe is standing out almost to its entire extent, but is held in place by the surrounding muscles of the eye. At the root of the nose bone necrosis about

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* This condition of red corpuscle agglutination and hemolysis is not infrequently found in the lymph follicles of the spleen and in the lymphoid tissue elsewhere in the infectious diseases, especially of children. It seems to be the condition precedent to the formation of the hyaline network first described by Recklinghausen.

1 inch in diameter exposes the dura. The scalp does not present any of the conditions described on face, neck, and hands, and other than a few scattered freckles seems normal. The feet and ankles are edematous, but the skin of body and legs is apparently free from all pathological conditions.

The wounds were painful to the end, and patient required morphia, gr. one-fourth every three hours for relief.

The mental condition of patient remained apparently normal, as she answered questions intelligently to the last. A few times before death she asked for food and drank several glasses of milk, though with some difficulty on account of the false opening into the mouth from below the lower lip. No paralysis or symptoms of nerve or cord disturbance noted. She grew steadily weaker and died this morning.

Autopsy, Long Island Hospital, July 6, 1902. Twenty-nine and one-half hours post-mortem. *Clinical diagnosis:* Melanoderma lenticularis progressiva. *Anatomical diagnosis:* Marasmus; melanoderma; ulceration of the skin of face; fatty degeneration of the heart; acute vegetative endocarditis (mitral); amyloid infiltration of the spleen, lymph nodes, and kidneys; cloudy swelling of the liver and kidneys; infarction of the kidneys; thrombosis of the common iliac veins; streptococcus septicemia; edema of the ankles.

Body: That of a girl 10 years of age; length 118 centimeters; slender; extremely emaciated; rigor mortis absent; slight post-mortem lividity of dependent parts; the hair of a tawny red color; the face is extensively disfigured by ulceration; the legs are partially flexed.

The face presents a loss of substance most marked upon the upper side, extending from the nose to the angle of the jaw, to the ear, and upward to the parietal eminence; the ulcer is of such depth as to involve the orbit and to extend backward to the temporal muscle; the left eye is collapsed; the left frontal bone at the root of the nose is eroded; at this point there is a cavity 3 centimeters across x 2 centimeters in depth, lined with dirty grayish-green material; the collapsed remains of the left eye are dislocated outward and downward; the zygomatic fossa of the left side is exposed and contains greenish necrotic material; the alæ nasi are extensively eroded; upon the tip of the nose is a brownish crust, and a similar heavier crust is upon the upper lip; upon the right side of the chin is an oval ulcer 6 x 3 centimeters, with thick edges, and a clean, pale pink, granular base exposing the floor of the mouth and the teeth; upon the middle of the forehead are heavy crusts, and about the outer canthus of the right eye and upon the right eyelid are smaller crusts. The skin of the right temporal region and of the right side of the face below the eye is for the most part white and glazed; the skin about the angle of the mouth is thin, glazed, and slightly puckered; elsewhere the surviving skin of the face is marked by dark brown macules from one-half to 4 millimeters in diameter, in places confluent and almost black; in addition are larger areas of irregular outline, with multiple brownish black foci. The deepest pigmentation and the most confluent macules are at the angle of the jaw and in the lateral cervical regions; the scalp shows brown macules, 1–3 millimeters across, with a frequency of two or three to the centimeter square; the skin of the back of the neck and of the front of the neck downward to the clavicle to the suprasternal notch presents numerous brownish macules; in the lateral cervical regions the macules are small.

The skin of the shoulders, the upper and forearms is thickly covered with very fine reddish hair; these regions are extensively pigmented upon the extensor surfaces, the forearms to a slight extent also upon the flexor surfaces; this pigmentation is in the form of pale brown macules, for the most part of the size of a pin's head, thickly set and but slightly confluent; the macules are larger than elsewhere upon the extensor surfaces of the forearms and upon the backs of the hands; the backs of the fingers show sparse millimeter-sized macules. The back of the left hand shows an area 3 x 1.5 centimeters, within which the skin is depressed, glazed, and slightly puckered; along this area are eight or ten horny outgrowths, from 3–12 millimeters in diameter and 2–4 millimeters in elevation, dry and yellowish brown; the skin of the backs of the fingers is somewhat atrophied, and that of the middle finger shows four or five warty nodules. The back of the right hand shows no definite areas of atrophy, but presents multiple warty nodules, mostly near the center.

The abdomen retracted; its skin normal.

The skin of the thighs and lower legs bears a growth of fine downy hair; that of the knees and the outer surface of the thighs presents sparse pale brown macules about 1 millimeter in diameter; the middle portion of both lower legs, both front and back, shows pale brown macules, rather sparse, 1 millimeter in diameter; in these regions the hair is most abundant; both ankles are edematous, the condition most marked upon the left, where it includes the dorsum of the foot. Decubitus of the sacral region and over the right trochanter.

The skin of the back from the roots of the hair to the upper borders of the scapulæ presents numerous brown macules similar to those present on the skin of the cervical region together with some atrophy; in the median line, at about the level of the shoulders, is a nodule 2 x 1.5 x 1 centimeters, freely movable and about one-third cornified.

Upon section, subcutaneous fat absent; muscles pale and thin; vermiform appendix and mesenteric lymph nodes normal.

Thorax: Height of diaphragm fourth rib on right side, fifth on left. *Pleural cavities:* Normal. *Pericardial cavity:* Normal. *Heart:* Epicardial fat absent; upon section, myocardium pale red and fairly firm; wall of left ventricle .8, of right .1–.2 centimeter in thickness; mitral valve 6, aortic valve 4.5, pulmonary valve 4.5, tricuspid valve 7.5 centimeters in circumference; depth of left ventricle 5.5 centimeters; the curtains of the mitral valve at the middle point of the line of apposition of each present pink, granular elevations within a region 6 x 3 millimeters and 2 millimeters in height; otherwise valves and cavities normal, the latter containing a small amount of blood, including a little white clot. *Lungs:* Fronts pale gray, backs pink; crepitant throughout with moderate edema; upon section, pink, moist, and crepitant. *Abdomen:* Peritoneal cavity normal. *Spleen:* Weight, 45 grams; general color pale bluish red; upon section, pale gray red; follicles visible as transparent, slightly violet-colored areas about .5 millimeter in diameter; upon application of Lugol's solution these areas become dark brown. *Gastrointestinal tract:* Negative; mesenteric and retroperitoneal fat absent. *Pancreas:* Normal. *Liver:* General color bluish red; upon section yellow brown, the lobules within certain areas are distinct, and here their centers are prominent and dark blood red; cut surface slightly opaque; consistence normal. *Gall bladder:* Normal. *Kidneys:* Weight of both 125 grams; capsule strips easily from a smooth surface; upon section, cortex .4 centimeter in thickness; cortex and pyramids gray, with radial red lines; glomeruli visible; iodine test negative. The right kidney upon its free surface at the lower pole shows two irregular areas about 2 centimeters in diameter, slightly elevated and yellow upon section, yellow near the surface and yellowish red near the pelvis. *Adrenal glands:* Negative. *Bladder:* Negative. *Genitalia:* Negative. *Aorta:* Normal. *Common iliac veins:* Both right and left contain partially obliterating thrombi, the proximal part white, the distal showing some central softening; on the right side the thrombus extends to the femoral vein, the proximal portion partially, the distal completely filling this vessel. *Cranium:* Not examined. *Spinal cord:* Not examined.

Cultures from heart gave streptococci.

The *bone marrow* showed a marked erythroblastic and slight myeloid activity. As in Case 616 there were very few lymphoid cells. The spleen showed complete amyloid degeneration and destruction of follicles, the amyloid often extending into surrounding tissue. There were also small strands of hyaline material and capillaries filled with agglutinated and partly hemolyzed corpuscles. A peculiarity in the case was the presence of foci of large epithelioid cells and giant cells somewhat resembling tubercle giant cells which were found sometimes in the center of and sometimes adjoining the amyloid. They lacked the caseation and other insignia of tubercles. A number of lymph nodes were examined and in all there was marked hypoplasia of the lymphoid tissue and germinal centers were absent. The lymph follicles of stomach and intestine show the same condition.

Sections of the mitral valve show slight acute fibrinous vegetations on a chronic sclerosed tissue. The myocardium is normal save for slight fat vacuolation. The kidneys show amyloid confined to the glomeruli and slight swelling and cloudiness of the epithelium of the convoluted tubules. Liver, pancreas, and intestinal canal show no changes. The thrombi in the iliac veins are in part recent, in part organized.

THE CUTANEOUS LESIONS. — Portions of skin from both cases involving all varieties of lesions were hardened in Zenker's fluid, sectioned in paraffin and celloidin and stained in a variety of methods. The stains found most valuable were the ordinary methylene blue and eosin, Mallory's connective tissue and phosphotungstic hematoxylin stain and the Verhoeff elastic tissue stain. In the two cases the conditions found were so similar that one description suffices.

In the epidermis there was everywhere a marked thickening of the horny layer. The thickening was irregular, the surface roughened by semi-detached fragments and shreds in which the single epithelial cells could sometimes be distinguished. Beneath this loose exfoliating surface there was a layer, often forming the main thickness of the epidermis, which was in part perfectly homogeneous and hyaline the cells so fused that they could not be distinguished, in part broken and fragmented into irregular layers. The granular layer was imperfect, only scattered cells with few granules being found, or it was absent altogether, the horny layer being directly continuous with the prickle layer. The prickle and germinal layers were in high degree atrophic, and represented by undifferentiated cells which were rarely more than three deep and were often limited to a single cell row. In places there was much similarity to infant's skin. Intercellular bridges were often seen between all of the cells, or were not at all apparent. The nuclei stained feebly, they were often shrivelled and irregular, the nuclear membrane being retracted from the nuclear space. The chromatin was small in amount and strongly clumped. In some of the cells a definite nucleolus, differentiated from the chromatin, was evident. In other places this nucleolus seemed to be covered up by the central clumping of chromatin around it. In places the nuclei were represented by feebly-stained large hyaline masses without evidence of chromatin. No definite nuclear fragmentation was found. No nuclear figures were found in the most atrophic areas, but there was evidence of direct nuclear division in peripheral clumping of chromatin, or various degrees of constriction of the nuclei. Three or more pale nuclei formed by direct division were often found in a single cell. It was hard to reconcile this extreme atrophy of the active cells of the epidermis with the great thickening of the horny layer and the constant exfoliation.

The projecting hard warty masses, so prominent a feature of the lesions, represented a local accentuation of the general condition of the skin. These were composed of dense adherent masses of horny cells and were often so hard that it was necessary to soften them in caustic potash before they could be sectioned. Some of them were long and pointed, others dome-shaped with a broad base. In some the cells united together forming hyaline structureless masses, in others the component scales could be made out. The scales were sometimes in parallel layers, in others in large whorls, and at the bottom definite epithelial pearls were often found. No vessel bearing papillæ projected from the skin surface into these warts; beneath them there were long papillæ which seemed to be due to a downward growth of the epithelium. The epidermis beneath these warty excrescences differed from that in the atrophic areas. The granular layer was often well marked both immediately below the wart and extending from it laterally. The epithelium beneath showed active proliferation. The cells extending to the corium were small with evident prickle borders. The nuclei were relatively large, contained abundant chromatin, and nuclear figures were numerous. The active cells formed a much thicker layer than in the normal skin. The mass of cells projected deeply into the corium and the base line was very irregular. The papillæ which were absent or small and blunt beneath the atrophic epidermis were here numerous and long. The edge of the epidermis gave off long prolongations, often branched and thin which extended irregularly into the corium, giving the appearance of a superficial carcinoma.

A striking peculiarity of the epithelial cells in these places was the extent of the fibril formation. The intercellular bridges were prominent and the fibrils forming them seemed to pass into the fibrillar structure of the cytoplasm, and the impression was often given that the fibrils passed continuously through or over the cell. In addition to these there were long fibrils straight or spiral in figure whose relation to the cells and nuclei was obscure and which seemed to pass through or between several cells. Some of the fibrils passed apparently through the cells and were in contact with the nuclei, others seemed to have no definite relation to the cells. The fibers varied greatly in size and length. In one place distorted spindle-shaped epithelial cells were found with long fibrils in the same relation to the cells as the fibroglia fibrils of the connective tissue. There was often a peculiar lateral extension of the growing epidermis. The layer of active cells pushed out beneath the adjacent atrophic cells separating these from the corium. There was a sharp line of demarcation between the brightly stained active cells and the atrophic cells above them, and in places an actual separation by spaces containing granular coagulum. In the numerous sections of the skin small areas of epidermic downward growth were found without any change of the surface. In these places small islands of epithelial cells having always the prickle character were found beneath the epidermis, but, as shown by serial sections, these were always connected with it. They extended from a point in the epidermis, growing out on all sides into the corium but never extending deeply into it. Polynuclear leucocytes were rarely found in the epidermis either in the atrophic areas or in the warty outgrowths.

The changes in the corium were as pronounced as those in the epidermis. As in the epidermis the most common condition was atrophy associated with various changes in the character of the tissue. The papillæ, except beneath the warts, were absent or small, blunt and atrophic. The layer of loose connective tissue just beneath the epidermis and which bears the blood vessels, was in places converted into a dense cicatricial-like tissue in which the individual fibrils could not be distinguished. This was connected with the thick tissue of the corium but differentiated from it. In one place in the subepithelial area there was a dense refractive hyaline network of tissue resembling thick masses of hyaline fibrin and which gave the reaction of neither connective nor elastic tissue. In other areas, particularly in the vicinity of the warts and tumors, there were small areas showing the same fibrinoid change which is so often found in the subepithelial tissue of mucous membranes in diphtheria. In places, this tissue was broader than normal, due to edema, and the epithelium was partially elevated from it, the usual granular coagulum enclosing small circles being found in the space. In other places this tissue was so thin and atrophied that the dense corium immediately adjoined the epidermis.

The elastic tissue of the corium was in general both relatively and absolutely increased. In places this increase was so marked that the thin corium seemed composed of it. These were the places where the entire skin was most atrophied, but on comparing this with normal skin taken from the same localities in children of the same age there seemed no doubt of the actual increase. In addition to this general increase focal masses of elastic fibers were often found just beneath the epidermis of the tumors and warts. The fibers in these whorls varied somewhat in size and gave the usual staining reaction. The mass in places seemed to disappear before the growing epithelium, there being a sharp boundary where this came in contact with it, in other places the mass of convoluted fibers was surrounded by the epithelium and became enclosed in it.

The fat masses in the corium were small, contained few and small fat globules, and had the general character of fetal fat. The nerves showed no changes. The arteries were few in number compared with the normal skin and were of normal structure. In but one place adjoining the ulcer of the face was an occluded artery found, the occlusion being seemingly the result of thrombus organization. The most common vessels were large, thin-walled veins which seemed to replace the capillaries. In places these were numerous and formed plexuses in the subepithelial tissue. Beneath the keratoses and the tumors the vessels were abundant. The hair follicles in the corium were abundant everywhere, the hairs were imperfectly formed, and composed of a dense mass without differentiation. No perfectly formed hair with a papilla was found. The cells of the sheath were small and frequently showed hyperplasia, the mass extending irregularly into the surrounding tissue. In no instance was this hyperplasia as marked as that of the epidermis cells, and even in the vicinity of the tumors it took no part in their structure. The coil glands showed degeneration consisting in atrophy with cell and nuclear fragmentation. This was most marked in the sections of skin which showed the most general atrophy. In sections from the back of the hand they were large, well formed, and without degeneration. The degeneration changes seemed more acute in these glands than elsewhere and in several cases polynuclear leucocytes were found among the fragmented cells. In one place in the vicinity of an area of rapidly-proliferating epithelium beneath a large wart, there was marked proliferation of the coils and of the duct nearest the surface. The coils were converted into thick cell masses which preserved an eccentrically placed lumen. The cells showed the characteristics of active cells and nuclear figures were found in them. There was no change in the deeper parts of the gland. The cells of the essential tissue of the corium as compared with the normal seemed fewer in number but they had the usual relation to the tissue bundles. There was in places a slight accumulation of lymphoid and endothelial cells around the vessels, but never to the extent found in other conditions involving such extensive changes. In the large dilated veins there was often swelling of the endothelial cells with evident proliferation though no nuclear figures were found in them.

Pigment was present to a greater or less degree in most of the sections even from places which to the naked eye showed none. In the most atrophic areas only an occasional pigmented cell was found. In the macroscopically pigmented areas a large amount was present but was never diffusely and evenly distributed as in the negro and in bronzed skin. The pigment was in the epidermis and in the upper layer of the corium and always in cells. Rarely was any found in the deep layer of the corium except in the most pigmented areas, where an occasional pigmented much-branched cell was found. In the subepithelial tissue it was contained in large cells of endothelial character which were generally in the walls of or around blood and lymphatic vessels. These deeply pigmented cells were found in contact with the epidermis. In the epidermis it was chiefly contained in the lower layer of the cells and in these in the greatest amount in the upper half of the cell nearest the surface of the skin. When present in large amount, the adjoining upper cells also contained it, and in several of the keratoses a similar pigment was found among the scaly cells. The pigment varied in intensity of color and was in the form of distinct granules, never in irregular clumps or in a crystalline form. It was of a bronze or greenish black color. There was no suggestion of the ordinary hematogenous pigment either in situation, form, or color. Nor were there any hemorrhages in the tissue or phagocytic inclusions of red blood corpuscles. In agreement with other observers we must regard the pigment as autochthonous in character, formed in the cells of the corium and from these cells transmitted to the epidermis.

Sections from an ulcer of the face showed the base of the ulcer composed of dense cicatricial-like tissue containing few cells and vessels. On the surface there was necrosis and foci of hyaline fibrinoid degeneration of the connective tissue. There was no edema, no infiltrating polynuclear leucocytes and no fibrin. Although elsewhere the skin lesions in no place extended beneath the corium, here the cicatricial tissue extended deeply into the underlying voluntary muscles, the fibers of which were atrophied and degenerated. On one side of the ulcer there was a downward growth of a thin epithelial reticulum, in which the fibril formation was marked.

The epithelial new formations taking the form of tumors were of the same general character in the two cases. There was but little difference save in extent of growth between these tumors and the epithelial growths in connection with the numerous keratoses. In all cases the tumors were superficial, they involved the epidermis and the corium only and were removed with the skin. The growth was most rapid in the upper parts of the tumors. Sections through the base of the smaller tumors showed small alveoli and small degenerating cells. In these parts the epithelial fibril formation was most marked. In Case 854 the alveoli were large, contained numerous pearls especially near the surface, and a relatively small amount of stroma which was densely infiltrated with plasma cells. In Case 616 the epithelial masses were smaller, the stroma more abundant, and the plasma cells absent. In neither case did the stroma of the large tumors contain any elastic tissue. In one of the small tumors in Case 616 single fibers and large whorls of elastic tissue were included in the alveoli. This was due to the masses of elastic tissue in the adjacent connective tissue being surrounded by and incorporated into the growing epithelial masses. The vessels in the stroma of the tumors were rarely of arterial character. They were large, thin-walled, and not very numerous. While all the other skin lesions rarely showed any polynuclear leucocytes these were very abundant in the tumors, appearing both as a general invasion of the epithelium and as large masses in degeneration spaces in the interior of alveoli.

SUMMARY. — Two female children, sisters, with the same definite clinical history of xeroderma pigmentosum died, one at eight, the other at ten years of age. In each case there was atrophy and pigmentation of the skin, the surface roughened and scaling and covered with warty keratoses. There were in addition large epithelial new growths which recurred on removal and extensive ulcer formation. The lesions were limited to the face, neck, shoulders, and upper extremities. There were in each case a few pigmented macules on the anterior surface of the lower extremities but no scaling or keratoses. In each case there was anemia, shown in one case by the clinical examination of the blood and in both by the examination of the tissues. In each case death could be attributed to the anemia and to a general malnutrition associated with and probably due to absorption from the ulcerated and putrefactive surfaces. At the autopsy in one case there was acute and chronic endocarditis of minor degree, amyloid degeneration and streptococcus septicemia. In both there was thrombosis of the large veins. The histological examination showed no disease save common degenerative conditions in the internal organs. The central nervous system in one case was normal, in the other it was not examined. In both cases the bone marrow was hyperplastic. There was distinct hypoplasia of the lymphoid system shown in the lymph nodes, the spleen, and intestinal follicles. In one case in connection with the amyloid there was a peculiar formation of giant cells in the spleen, the significance of which is not understood. The most common condition in the skin consisted in atrophy of the corium and its appendages, and of the epidermis combined with an excessive formation of the horny layer, and the focal formation of pigment. The atrophy of the corium was at the expense of the connective tissue. The elastic tissue showed a general increase and a focal formation of large masses of convoluted fibers. In places there were horny excrescences and tumor-like masses of the epithelium. The tumor formation did not penetrate below the corium and there were no metastases either in lymph nodes or internally. The pigment was intracellular and autochthonous.

EPICRISIS.—The chief interest in these cases attaches to the etiology, to the skin lesions and to the relation of the tumor formations to these lesions. In regard to the etiology it would seem that we must assume a congenital condition in consequence of which these children are more susceptible to the action of a causative agent. In favor of the presence of such a congenital condition is the family relation of the disease and its appearance in the family in children of the same sex. It has generally been assumed that this supposed congenital predisposing condition is attached to the skin, but this is not necessarily the case. In the intimate interrelation of tissues it would be perfectly possible for the nutrition, function, and reaction of the skin to be disturbed by some organic or chemical disturbance in the interior of the body. No evidence of such a congenital change obtained from the skin itself has been brought forward. In these cases the skin of the unaffected areas appears perfectly normal. Assuming such a general predisposing condition the direct etiological agent would probably be one acting only on the parts of the body which are affected. For although not impossible it would be in the highest degree improbable that a congenital condition would only affect certain areas of the skin in all cases. The most probable direct etiological agent is sunlight. The strongest evidence in favor of this is the areas affected, and if the disease were limited to the face, neck, and hands the evidence would be very strong. While the lesions are most marked here they also extend over the chest, arms, and shoulders, and to a very limited extent over the lower extremities, and these parts certainly seem to be as well protected from light as other parts of the body except in infants. There is also evidence in the literature of exacerbations of the disease following directly upon exposure to the sun. In Bronson's case the face was swollen and red after exposure to the sun, and this lasted several days. In one of Lukasiewiez's cases which was observed for two years there was redness and swelling of the skin following exposure to the sun except in the atrophied whitish areas. As the atrophied areas increased in size the areas of erythema became smaller and smaller. The areas affected follow pretty closely the ordinary distribution of freckles of the skin. It is also possible although some of the areas are as fully protected by clothing as the unaffected parts, the shoulders and arms are more directly exposed to sunlight than other parts, the rays in the ordinary positions of the body striking them more directly. We must remember, however, that in small-pox the eruption first takes place and is most marked over the parts of the body affected here, and it would be difficult to ascribe this to light action. One would think the disease should be more common in the tropics especially among the English residents, for the natives would have the protection of the skin pigment. However, little value can be placed on the statistics of the disease for, through its rarity, it is not so known to physicians as to be generally recognized. The pigment formation which is so marked a feature is probably an effect of light, for there is an undoubted relation between pigment formation and light. Although the disease is more common in blondes there have been cases in marked brunettes. Another point in favor of light action is the similarity of the skin lesions to those produced by the X-rays. In these the essential conditions are atrophy, roughening, and scaling of the surface, the formation of keratoses and tumors. The X-ray tumors are epithelial in character, superficial, non-invading, and there is little tendency to metastasis. Zieler has performed an experiment which throws some light on the etiology. In a case of xeroderma reported by Hahm and Weik, they exposed a small unpigmented area on the thigh to the rays of a Kromayer quartz lamp for twenty minutes. There was a strong reaction with necrosis of the papillary body, which after some weeks healed. Pigment then developed between the edge and the middle of the scar. Microscopic examination showed a typical scar without papillæ with pigment in the lowest cells of the epidermis and in the form of large clumps in the cutis. Little attention has been paid to the blood, and it is possible that this may have some bearing on the disease. In both of our cases there was marked anemia, and also in three cases studied by Okamura. There was also at autopsy a marked hypoplasia of the lymphoid tissue and in the one blood count made a reduction of the lymphocytes. There is no adequate explanation of this condition. Okamura thinks the anemia and reduction in hemoglobin which he observed was secondary to the skin affection and due to disturbance of the general nutrition.

Of the skin lesions the atrophy seems to be the essential condition. It affects all parts of the skin, corium, epidermis, and epidermic appendages. The atrophy could not be referred to imperfect nutrition following vascular disease. The vessels were few in number and they seemed to share in the general atrophy. There was no true hyperemia even in the parts which to the naked eye appeared reddened, for the vessels here were merely large, thin-walled veins. The general non-vascularity was shown by the absence of granulation tissue with newly-formed vessels in the bases of the ulcers. Where, however, there was actively-growing epithelium as below the keratoses and the tumors there was an abundance of vessels. With the atrophy of the corium there is associated a new formation of elastic tissue which in part is of the character of normal elastic tissue, in part forms large masses of convoluted fibers. No light was thrown on the manner in which this new formation took place. In other places elastic tissue formation seems to be a secondary phenomenon to primary injury and degeneration and it should here be probably so regarded.

Are the tumors to be regarded as true tumors? They have not the characteristics of unlimited local extension and metastasis formation. There is a gradual extension from small down-growths of epithelium to the keratoses and the tumors. The only distinction between the tumors and the warty keratoses was the abundance of horn formation in the one and the more rapid epithelial growth in the other. All were atypical and all represented stages of development. We know the frequency with which an epidermoid carcinoma starts as a keratosis. We must regard the large epithelial masses as conforming to the most marked characteristics of the epidermoid carcinomas of the skin.

In our cases there was nothing on histological examination to separate the tumors from ordinary epidermoid carcinoma of the skin, except when the bases of the small tumors were examined. Instead of finding these lowest cells the most actively growing they were small and atrophic, and the growth never extended below the corium. With few exceptions all the tumor formations described in the disease have been epidermoid carcinomas. Pick's case of melanotic sarcoma of the leg, and the case of Hanke, in which a small tumor removed from the eyelid was described as perithelial sarcoma, probably had no relation to the disease. Philip has analyzed all the cases of carcinoma in childhood which he could obtain from the literature and finds most of the epidermoid cancers to be in connection with xeroderma. Cases are very rare up to the seventh year, when there is a sharp rise reaching a maximum at nine, then decrease with sharp rise to total maximum at twelve. The period from seven to nine corresponds with the tumor development in xeroderma. There is no tendency to the formation of sarcoma or carcinoma elsewhere in the body.

In what relation do the tumors stand to the disease? We need here pay no attention to the question of parasites. It would be difficult to think of a parasite which could find only in this disease the suitable conditions for development. The tumor formation is not an early but a late manifestation in the disease. If it were due to the action of the same etiological factor which produces the disease, we should expect it to occur at any time. The tumor formation follows the atrophy and other lesions of the skin here in the same way it does in the case of the X-ray tumors. In pathology, there are instances too numerous to be cited in which epithelial growth definitely follows on changes in the character of the underlying connective tissue. That these common atypical epithelial growths do not go on to unlimited growth and tumor formation is probably due to the fact that in interruption of the normal mutual relations between tissues local restrictions to growth are removed and the growing epithelial cells have only the normal growth capacity. The repair or regeneration of the subjacent tissue may quickly put an end to the excessive epithelial growth. Further there is a strong analogy between the xeroderma tumors and the epidermoid cancers which develop in the atrophic skin of old people, as there is between the whole series of skin changes in xeroderma and senile atrophy. We think on the whole that the evidence is that the epithelial growths follow upon and are dependent on the atrophy and other changes in the skin. Why there are these foci of epithelial growth with epithelial atrophy elsewhere we do not know, but it may depend upon the character of the changes in the corium. The epithelial atrophy is associated with a dense cicatricial corium; where the epithelial growth takes place the tissue though atrophic is looser. It may be that the factor in atrophy which leads to epithelial growth is merely mechanical, the opposition of tissues being removed, or it may be that in the process of atrophy chemical substances which stimulate growth are formed or those which inhibit growth may be used up and removed. The influence, whatever it is, is local and only locally has the epithelium this great capacity for growth. Differences in the vascularity will not explain it. Nothing in pathology is better established than the saying of Virchow that the tissues are not nourished but nourish themselves. Active growth of tissue is associated with vascularity and we do not understand how the interrelation is brought about, but vascularity will not of itself produce growth. The absence of metastases shows that the individual cells of the tumors do not carry with them the same capacity for growth which they exhibit locally. We see the same absence of metastases in many of the senile epidermoid cancers and in most of the cases of X-ray cancer that have been reported.

From all that we know of this most interesting skin disease it would seem that successful treatment would depend on its early recognition and the careful exclusion of the skin from the action of light, probably the violet end of the spectrum being the most injurious. Whether any form of applications to the skin would have any efficacy seems doubtful. In the future observation of cases attention should be directed to the anemia and a careful study of all the reactions of the skin not only in the areas affected but elsewhere. Small areas should be exposed to sunlight, to the different rays of the spectrum, and to the X-rays and the effects carefully studied by all methods. The disease is of great importance, for its future study may lead not only to greater knowledge of the obscure subject of the injurious effects of light but also of the questions of growth and tumor formation.

DESCRIPTION OF PLATES.

PLATE XXXII.

FIG. 1. — Case No. 1 (616) photographed a short time previous to death.

PLATE XXXIII.

FIG. 2. — Photograph showing proliferation at base of a keratosis and the sharp separation of the proliferating epithelium.

FIG. 3. — Atrophic area of the skin.

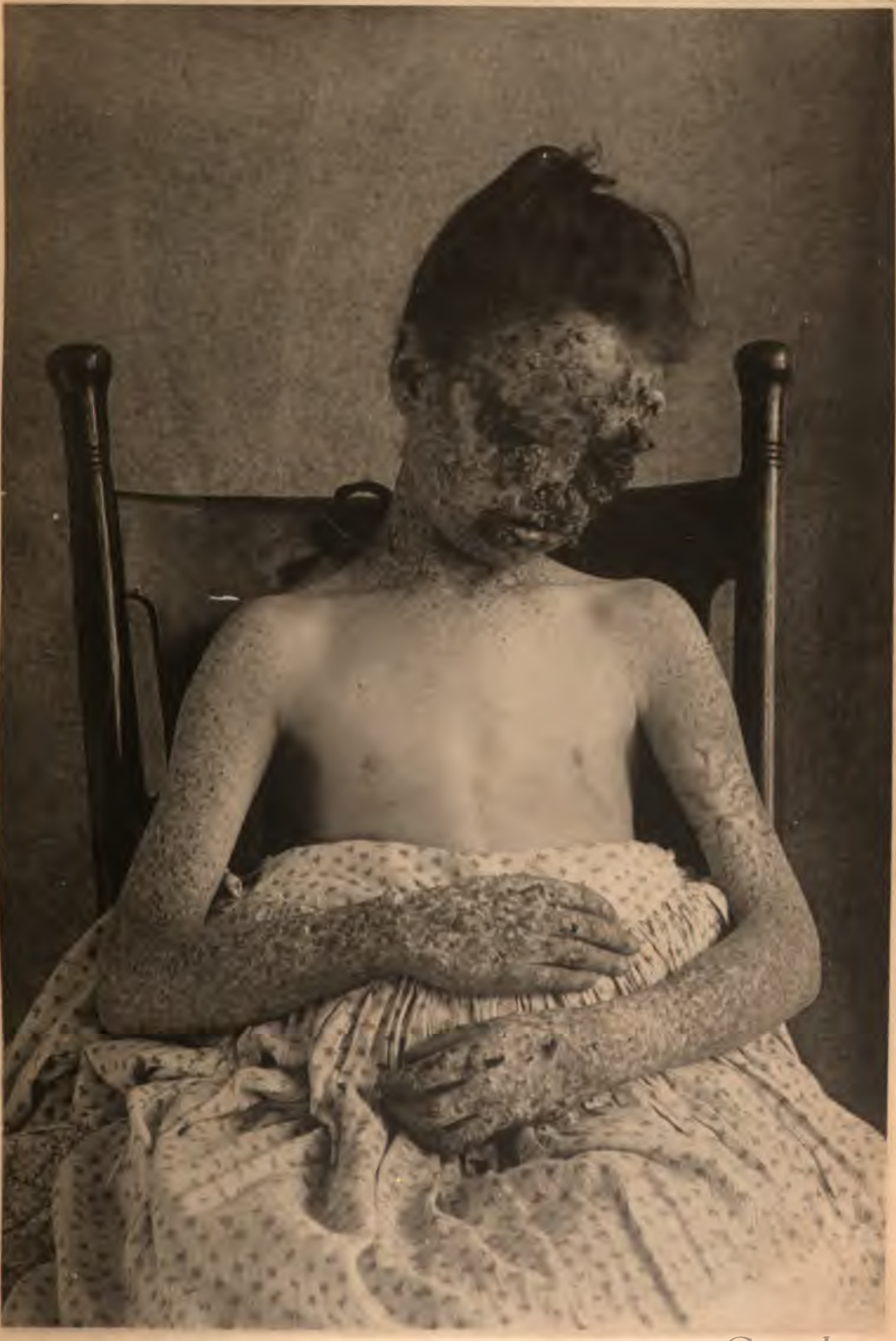
FIG. 4. — Edge of keratosis showing the lateral outgrowth of proliferating cells.

PLATE XXXIV.

FIG. 5. — Hair follicle showing proliferation of the cells of the sheath.

FIG. 6. — Masses of elastic tissue in corium adjoining an epithelial downgrowth.

FIG. 7. — Masses of elastic tissue included in epithelium.



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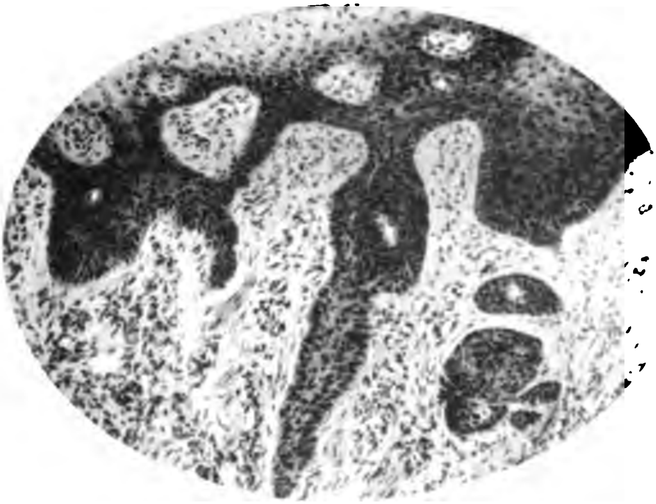
Councilman and Magrath.

Xeroderma pigmentosum.



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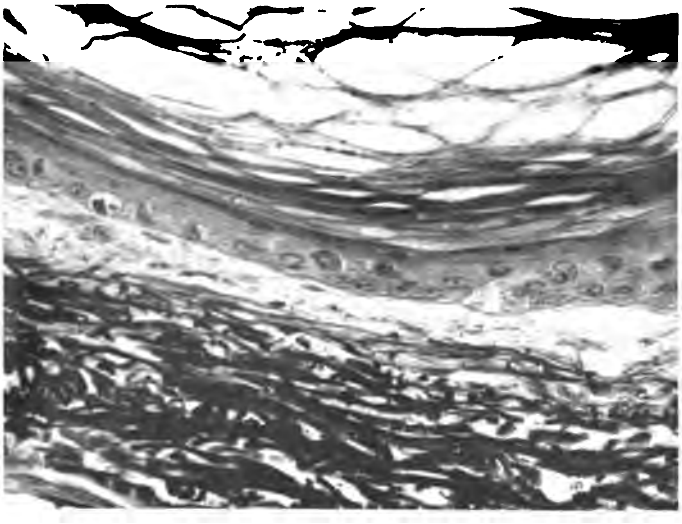

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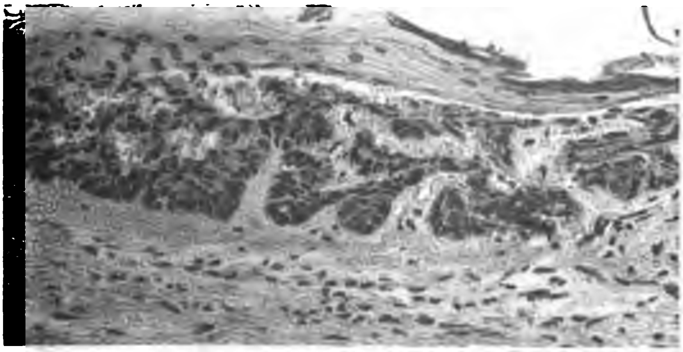

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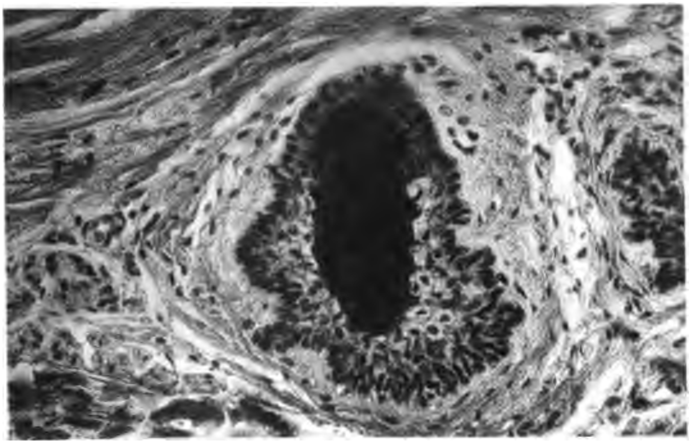
Councilman and Magrath. Xeroderma pigmentosum.



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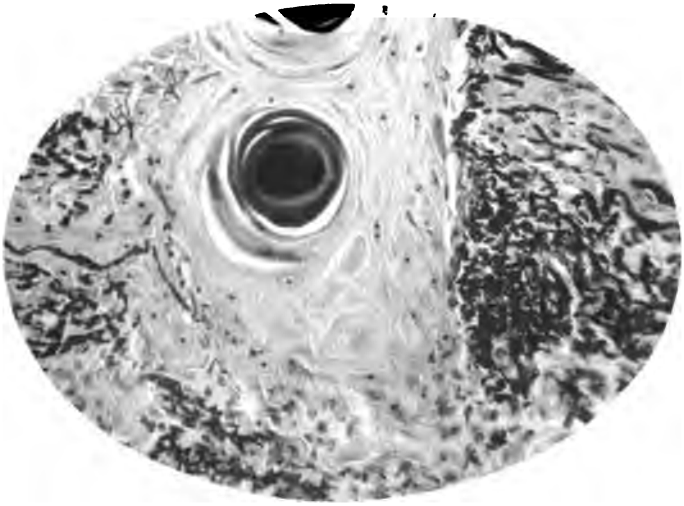

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7

Councilman and Magrath.

Xeroderma pigmentosum.

THE SURGICAL TREATMENT OF X-RAY CARCINOMA AND OTHER SEVERE X-RAY LESIONS, BASED UPON AN ANALYSIS OF FORTY-SEVEN CASES.*

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In the Annals of Surgery for November, 1907, I published the account of two cases of severe X-ray lesions; one of possible sarcoma of the finger, the other of an X-ray operator who, since 1896, in addition to the severer forms of dermatitis and ulceration, had developed, between 1902 and 1907, numerous carcinomata, requiring many operations, amputations, excisions, and skin graftings. At the time, I was able to gather the records of ten other cases making, with my own, eleven undoubted X-ray cancers, of which six had been fatal.

Since that time occasional reports of cases have appeared, and three notable papers by E. Schümann[1] in the Beiträge für Klinische Chirurgie, 1907, LXXXIV., Heft 3, page 355; by Karl Lindenborn,[2] in the same journal, 1908, LXXXIX., Heft 2, page 385; and by Dr. Cecil Rowntree[3] of London in the Archives of the Middlesex Hospital, XIII., July, 1908. In all of these papers new cases have been presented, and the etiological relation between X-ray lesions and carcinoma, possibly also sarcoma, has been thoroughly discussed and, in the case of the former at least, definitely proved. In the German literature especially, the subsequent development of cancer upon lupus tissue treated with X-rays has aroused considerable interest; though Steinhans has collected eighty-three cases of carcinoma following lupus, without X-ray treatment, it seems, after a careful perusal of the histories, at least probable that the X-ray had a definite influence upon the subsequent development of the cancer. The cases can be studied in detail in the contributions of Schümann and Lindenborn.

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* Received for publication July 26, 1909.

I propose in this paper to omit all consideration of the lupus cases, and to limit myself, with very few exceptions, to a consideration of the severer lesions which have resulted from the prolonged action of the X-ray upon previously healthy skin, and I should like to emphasize the fact that not only was the skin healthy, but the wearer of it was almost without exception young.

As a result of my former publication, I have had, so far as I know, a unique experience, having operated upon eleven other cases in addition to further operations upon both of the cases previously reported. Thirteen in all: of these three were non-malignant, two beginning carcinoma, six carcinoma, one sarcoma(?), another carcinoma or sarcoma. The distinctions between the phrases precancerous and beginning epithelioma must of course be a matter of opinion, but the clinical histories of other similar cases in this X-ray series seem to show that tissues which presented a precancerous condition or commencing epithelioma at the time of excision would, unless adequately treated, subsequently develop into squamous celled carcinoma. From my experience it seems almost assured that, given lesions of a sufficient duration and severity without adequate treatment, the future development of cancer can be predicted.

Though the harmful results of continuous exposure to the X-rays were unknown to the early workers in this field, it would seem that unwittingly they have given us the best demonstration yet known of the artificial or experimental production of cancer. It is unlikely that old age itself, with its accompanying skin atrophies, even if combined with exposure to such various noxious influences as sea life, raw winds, powerful actinic rays, soot or paraffin, would give such an example of malignant skin degeneration as seems so frequently to result from protracted exposure to the X-ray. When it is remembered that these lesions have been produced in young men at an age when skin cancer is extremely rare, its occurrence is all the more striking.

I have gathered from the literature, and especially through the kindness of physicians whose names will be mentioned with their personal communications, combined with my own experience, the records of forty-seven cases of severe X-ray lesions. This number in no way represents the actual figures, for there are surely very many cases both of serious damage and also of carcinoma which have not been reported, and are therefore not available. For example, Dr. Pusey of Chicago states:

> "I have seen many cases of this kind; certainly more than a score. They are all of one kind and may be briefly characterized in a group. As a result of either an acute or more frequently of a chronic long continued X-ray dermatitis, the skin becomes atrophic, thinned, and harsh, with a number of dilated blood vessels coursing through it and many keratoses forming upon it. These keratoses very frequently become the site of epithelioma. In my judgment, the condition is analogous to senile atrophy of the skin and on the basis of this analogy I prophesied, before the report of any epithelioma in these cases, that epithelioma would develop in them. At the start these X-ray epitheliomata ought theoretically to be very easily handled, just as the superficial epitheliomata beginning in senile keratoses are very easily handled and this has proved true in my experience. In my judgment these epitheliomata should be handled in exactly the same way as epitheliomatous senile keratosis, either by excision or by some other destructive procedure. Many such lesions have been excised upon my advice with good result. Many others that were very superficial I have treated by destruction, usually with carbon dioxide snow. The reason for this sort of procedure is that it does not require the destruction of as much tissue as a thorough excision and sometimes, of course, this is a matter of importance."

Dr. Hyde, in addition to the cases recorded, says that he has seen at least six other patients in a precancerous stage. The experience of these dermatologists is, no doubt, equalled by some in this and other countries, so that the cases reported are by no means an index of the number of persons who are now suffering from early exposures. From France, so far as I am aware, there has been no report of serious X-ray lesions.

Of these forty-seven cases, four of my own were not unusual and are reported simply for record. Four were described as in a precancerous stage or beginning epithelioma; thirty-six were undoubted epidermoid cancer, and two of my own, questionable sarcomata. Had it not been for the reports of sarcomata by other observers, especially in conjunction with the X-ray treatment of lupus, these two cases would be open to more question. As it now stands, it seems probable that the X-rays may be a factor in the production not only of cancer, but of sarcoma. Of the thirty-six cases of epidermoid cancer, nine have died; a mortality of twenty-five per cent, showing I think quite clearly that these X-ray carcinomata are as malignant, if not more so, than the epitheliomata which ordinarily grow in the skin of older persons, and are, if allowed to advance beyond a certain stage, prone to early metastasis.

In the histories of these cases, the reader will be struck by the period of incubation, if so it may be called, which dates from the occurrence of the original severe lesions, or early unprotected exposures to the rays, during which time characteristic changes have taken place in the skin and underlying tissues, which precede the development of a cancer. This period varies from three to eleven years (Case XXII.), usually from five to seven, and is dependent no doubt upon the number of the exposures and the vulnerability of the individual.

While progressive and characteristic lesions have developed since the dangers of the X-ray were recognized, and some precautions were taken, it is among those who did the earliest work that we have had the greatest number of fatalities and the most severe lesions; and this without regard to the middle age or youth of the individuals. It has seemed that the development of the photographic plates combined with X-ray work has led to earlier and more serious lesions than when X-ray work was done alone. We have, then, quite clearly differentiated from other skin diseases a definite occupation dermatosis which can be diagnosed at a glance, and in the chronic form resembles no other skin affection known to me. The dry thickened skin, without hairs, the telangiectases, the ribbed nails, the keratoses on the backs of the hands in young men are together pathognomonic, and show the results of the specific action of the X-ray; in a more advanced stage, paronychia, more numerous keratoses, rhagades and ulcerations which alternately heal and break down, until finally there comes, as inevitably as fate, the development of epithelioma at the base of a persistent or inflamed keratosis, or in a chronic ulcer. While the keratoses in no way differ from the usual senile condition, the combination of juvenile keratoses with ulceration of the skin of the dorsum of the hands and fingers seems peculiar to X-ray dermatoses. I have not yet seen an X-ray lesion of the palmar surface of the hands or fingers. Either the thickened skin offers some immunity, or what is more probable, the dorsum, from the position of the hands in work either with the fluoroscope or otherwise, is more exposed. The base of the middle finger seems to be the place most universally affected. This knuckle is obviously most exposed and nearest to the tubes. The thumb, while the nail and the ulnar border is occasionally affected, is to some degree protected in most exposures by the thick palmar skin. The nails suffer relatively early, and the changes are to some degree permanent. An accidental discovery in the case of J. G., Case XVIII., seems to show the value of protection during the early years of work, and the lack of harmful influence to recent exposures with proper precautions. A broad gold ring was worn during the first two years of work on the ring finger of the left hand. This was subsequently removed. The whole dorsum of the hand shows the characteristic changes, while the skin protected by the ring remains to this day perfectly normal. The immunity which even light clothing offers is shown by the rarity or slight degree of dermatitis above the cuffs, and in those parts of the body protected by clothing. It would seem, therefore, in view of this immunity from slight covering, that not the X-ray themselves, but other emanations from the tube are to be held chiefly responsible for the burns and the chronic dermatitis. With these few general remarks, I shall proceed to record the cases, with the pathological reports when available, and to give after each one a brief summary in which any interesting feature will be mentioned.

CASE I. — Miss A. Personal communication from physician. Owing to slight tuberculous disease of the ankle, patient was subjected to vigorous X-ray treatment for a year and a half. Extensive X-ray burn resulted in an intractable deep ulceration 3 x 2 inches in extent, just above the ankle on the inner side of the leg. Pain was extreme. Amputation at point of election, wound healing, but pain in stump persisting a year after operation. No evidences of carcinoma in the ulcer.

Comment: Excision and skin grafting might have been done in this instance but, owing to the tuberculosis of the tarsus, amputation seemed preferable.

CASE II. — Personal. Dr. Wolbach, Case I. F. M.; age 55; was operated upon for adenocarcinoma of the stomach, with partial gastrectomy, Dec. 19, 1905; recovery was perfect. On July 23, 1908, after swallowing bismuth, he was examined for not more than twenty minutes with the fluoroscope, the tube being supposedly at a distance of fifteen inches. In three weeks he reported complaining of severe pain and an area of erythema about the size of a dinner plate, in the middle of the back. This was treated with one per cent. ichthyol wash, but with extreme suffering, the skin broke down, forming an irregular ulcer about three inches by two, which was resistant to all treatment, and steadily increased in size.

Operation under ether, Nov. 18, 1908, Massachusetts General Hospital. In the center of the back, over an area 4½ x 3½ inches, is a superficial ulcer, surrounded by an irregular margin of reddened skin, situated over the last two dorsal and first two lumbar vertebræ. The base of the ulcer is slightly depressed, showing no evidence of granulation tissue, but the surface is covered with firmly adherent fibrin. There are several roundish, moundlike elevations, varying in diameter from a quarter to a half inch. After careful preliminary cleansing, the whole area, with a margin of three-quarters of an inch, was excised down to the underlying muscle. The choice then arose as to a plastic operation or a skin graft; the former was chosen. A flap was made with the base uppermost, extending downward and to the right. This was dissected up and turned over, and sutured without tension to the margins of the wound. Another flap was swung from below into the denuded area; approximation was almost complete. In a few days the wound became infected from a nearby furunculosis and, in spite of vaccines, there occurred some sloughing of the distal part of the flap. Healing took place slowly, but without fever. Superficial necrosis of the surrounding skin is still taking place, and raw surfaces being covered with a characteristic fibrinous membrane. On December 22, the patient was temporarily discharged from the hospital, the depth of the wound granulating healthily, but the skin still undergoing superficial necrosis.

Summary: X-ray burn after long exposure, July 23, 1908. Ulceration began six weeks afterwards, steadily progressing with great pain in spite of treatment. Excision of large ulceration on back on Nov. 18, 1908. Operation brought about immediate and permanent relief from pain, but subsequent necrosis of the edges of the wound raised the question as to whether the excision had been adequate. Whether the sloughing of the flap was due to infection or to inadequate blood supply, on account of the obliterative endarteritis present, is an open question. There are other instances of the sloughing of flaps where the skin has been for a long time exposed to X-rays. This at least would serve as a warning in the performance of plastic surgery in these cases, and were I to operate again I should simply excise such an ulcer with primary skin grafting. The mounds mentioned on the surface of the ulcer were shown by microscopic examination to be blisters in the deep layers of the epidermis.

Pathological report by Dr. Wolbach. Material consists of Zenker hardened tissue representing a large ulcer about 10 centimeters in diameter surrounded by a border of epidermis 1 to 1½ centimeters in thickness.

Microscopic description: paraffine sections; phosphotungstic and hematein stain.

The portions covered by skin are very similar in appearance to the tissue from the cases of Miss H. and Mr. S., that is, we have the same rarefication of the corium immediately beneath the epidermis. The papillæ are absent, so that the epidermis presents a perfectly smooth under surface. The portion denuded of epithelium shows a slight fibrinous exudate overlying the corium. In a few places there is granulation tissue beneath thick layers of coarse meshed fibrin. The epidermis in places is markedly thinned and the ordinary arrangement of the basal layer is lost. The cells of the epidermis in the thin portions are larger, somewhat vesicular in character and stain less deeply than those of normal epidermis. There is nowhere any evidence of invasion of the corium by epidermis. The deep vessels of the corium show very slight changes. There are many coil glands which are practically normal. Others are atrophic and are surrounded by new formed connective tissue and masses of lymphoid and plasma cells. No hair follicles or sebaceous glands found in the section. Irregular areas and tracts throughout the corium filled with lymphoid and plasma cells are probably spaces once occupied by coil glands and ducts.

In brief, this tissue shows the same processes found in Case VIII. and Case IV., but to a less advanced degree. The characteristic changes of the corium are present. Vessels show very slight thickening of the intima. Epidermis, however, does not show irregular downgrowth suggesting beginning carcinoma.

CASE III. — Personal. Dr. Wolbach, Case II. E. R.; 28 years (female); referred to me by Dr. Reeves of Boston. Patient consulted Dr. R. first for ulcerations of elbow and knee on March 18, 1908. X-ray treatment until the end of April at the Emergency Hospital, when they broke down and she went to the Emergency as a patient for three weeks, as ulcers formed on both knee and elbow. Under treatment, the acute dermatitis subsided, but since then the ulcers have never healed. While at hospital the ulcer diminished on the knee from 5 x 3 inches to 2½ x 1; that on the elbow from 2½ x 1½ to the size of a "quarter." Patient worked for two weeks, and although the ulcerations did not increase in size, the skin surrounding them became irritated. Was again admitted to hospital for three weeks in June; improvement under rest and treatment. Reëntered August 18, where she remained until Nov. 7, 1908, when she was discharged. Daily dressing since then.

Examination Dec. 4, 1908. Over inner aspect of flexure of elbow are characteristic X-ray changes; evidences of old ulceration about 4 x 3 with cicatricial tissue and telangiectases. Just above the inner condyle is an ulceration about size of a dime which is covered with a yellow, fibrinous scab. The skin about it is atrophic; causes pain on motion; no dermatitis. Examination of left leg: dermatitis from middle of thigh to top of boot; a great deal of irritation and itching. There is some edema. On the outer side of the knee over an area 6 x 5 inches the skin is reddened, pigmented and shows scars peripherally to the old healed ulcerations; also telangiectases. Ulceration at present is 2½ x 1 inches; sore and tender to touch. Pain and sensitiveness, however, are less now than during acute inflammation. Patient says boric acid, aristol, corrosive wash, as well as ichthyol all irritated the skin. The edges of the skin show little evidence of healing; there is fibrin at the margin of the growing epithelium. The whole skin of this area is indurated and is two or three times the normal thickness. There is already some tendency to cicatricial contraction, but this is on the outside of the knee, not in the popliteal space. The dermatitis of the rest of the leg can be properly called medicamentosa.

Operation, Jan. 21, 1909, N. E. Baptist Hospital. Since last visit, the patient has been comparatively free from pain, as the dermatitis has subsided, but the ulceration shows no evidence of healing or of growing smaller. Its base is very much indurated. Under ether, thorough excision down to fat, with half-inch margin. After bleeding had stopped raw surface was covered with Thiersch grafts from the left thigh. Protective tissue; firm pressure; splint.

Jan. 28, 1909. First dressing. Little maceration; five-sixths of the grafts have taken, but here and there in the edges, and in one or two places in the center, there have been small hemorrhages with resulting necrosis.

Jan. 30, 1909. Grafts exposed to air and apparently doing well.

Feb. 24, 1909. Almost all of the grafts took, but here and there, one or two small areas subsequently broke down and showed a tendency to spread. It would seem to me as if excision had been inadequate and further operation was advisable. This proves to me quite clearly, in connection with the S. case, that it is hard to gauge how much devitalized tissue should be removed; although my excisions have appeared at the time free enough, I think in general they may have been inadequate.

March 25, 1909. As there had been a previous history of specific disease in this case, I decided to try the effects of internal treatment before resorting to further grafting. After ten days of mixed treatment and the local application of black wash, the broken-down areas were entirely healed over. It would thus seem as if the original ulcerations, for which the X-rays had been applied, were undoubtedly specific, and that in spite of grafting the disease progressed from a lower level.

April 1, 1909. Patient is entirely free from pain. Grafts are beginning to be mobile; the leg is soundly healed.

For pathological report see Dr. Wolbach's paper under Case II.

CASE IV. — Personal. Dr. Wolbach, Case III. F. H. S.; 40 years; referred to me by Dr. C. J. White Nov. 16, 1908. Treatment with X-rays five years ago, for eczema of both legs, with marked improvement. After subsequent, rather frequent treatments, extending over a period of a year, there developed a rather severe dermatitis which healed slowly with complete desquamation of the superficial layers of the skin from the middle of the thighs downward. Four years ago there appeared on the inner side of the left calf a small ulcer, which from that time has never completely healed. On the right shin there was also an ulcer which took months to heal. Both popliteal spaces showed characteristic telangiectases. There has been no pain. In August, 1908, the ulceration of the left leg began to spread rapidly and refused to yield to any treatment; radium was twice applied. Examination on Nov. 16, 1908, shows about the middle of the left leg an ulceration, 3 x 2½ inches. The edges are irregular, slightly indurated; in some parts there are islands of growing epidermis, and in others fairly healthy areas of granulation — in still others, there is an unhealthy surface covered with adherent fibrin. The skin is undermined and shows no evidence of repair. Operation was advised and accepted.

Operation under ether, Nov. 19, 1908. Leg was disinfected with extreme care and the whole ulcer excised with a margin of half an inch down to the subcutaneous fat and under the old ulceration to the sheath of the gastrocnemius muscle itself. After bleeding had ceased the wound was again disinfected and moderately thick skin grafts were taken from the left thigh. These were stitched to the edges and wound covered with protective and a firm bandage. On the evening following operation the temperature rose to 101, but he had no pain. At noon on the following day the wound was dressed, the temperature being at that time 100. It was found that infection had taken place, and that the periphery of the grafts had sloughed with some hemorrhagic infiltration into the central portion. In spite of exposure to air, alternating with antiseptic fomentations, all of the grafts separated, except for an irregular area about three-fourths of an inch in diameter, in the very center of the wound. At the end of two weeks the wound became healthy, and granulation was well established. Small Thiersch grafts from another patient were placed on the surface, but in two days all of these except two had likewise sloughed; in the meantime there had been an unusual amount of suppuration of the thigh from which the grafts had originally been removed.

The lower edge of the wound, where the skin was twice the normal thickness, showed no evidence of healing. While infection probably played the chief role in this failure, it seemed to me not unlikely that the tissues both of the thigh and of the leg were less resistant owing to the previous X-ray injuries. On several occasions, small strips of skin were placed upon the wound and covered with crêpe lisse. These grafts with the rapid growth of the original Thiersch graft in the middle allowed the patient to return to his home on Dec. 22, 1908, with the wound four-fifths healed.

Pathological report by Dr. S. B. Wolbach. A large piece of Zenker hardened tissue consisting of an ulcer with surrounding skin. Microscopic examination: Zenker fixation; paraffine sections; phosphotungstic acid-hematein stain.

The tissue is very similar to that from the case of Miss H. The ulcerated surface shows typical granulation tissue springing from a base of rarefied corium. On the surface are small islands of epithelium which present no striking or unusual characteristics. The most striking changes found in the sections are in the corium just beneath the epidermis surrounding the ulcer and in the vessels throughout the sections. The connective tissue cells of the corium just beneath the epidermis are widely separated and of embryonic type in appearance; they are surrounded by very few delicate collagenous fibrils. In this rarefied corium are many large blood vessels lined with a single layer of endothelium. A few of these vessels lie in direct contact with the basal layer of the epidermis, some are completely surrounded by epidermis and a few are filled with fibrin thrombi.

In the deep layers of the corium there is marked thickening of the walls of both arteries and veins. The thickening is due chiefly to an increase in the connective tissue of the intima. The endothelium of some vessels is swollen. There is also an increase of connective tissue in the muscular coats. There are numerous completely obliterated arteries.

The coil glands are nearly completely absent. A few atrophic tubules are found in areas of new formed connective tissue. No hair follicles or sebaceous glands are to be found. The epidermis surrounding the ulcer is slightly thickened. The horny layer is strikingly narrow and in places absent. There are many irregular downgrowths of epidermis into the rarefied corium, but these processes all present an orderly layer of columnal basal cells. At the edge of the ulcer are downgrowths of atypical epithelium, not however more striking than in the case of any chronic ulcer.

There is nowhere any suggestion of malignancy or actual invasion of the corium.

Subsequent history: Feb. 1, 1909. Upon returning to his home, the wound was completely healed in three or four days, but on January 2 the grafts which had been taken from another patient slowly broke down with the formation of blisters, and upon his arrival in Boston had entirely disappeared. The under surface, however, was healthy, and in two days snips were applied from his arm and covered with crêpe lisse. These grafts rapidly coalesced by January 20, and he was discharged home entirely healed and has so remained until the present time.

CASE V. — Personal. W. H. M. (physician), Lawrence, Mass.; 43 years. Patient's hands have been treated by the X-rays for seven years, for eczema; exposures ten to fifteen minutes — both coil and static machines. Extensive, rather superficial burns of both hands in January, 1906; the skin sloughed off but was replaced. Later exposures in May, 1906, followed by slight dermatitis. Last exposure, Dec. 10, 1906, static machine, five inches, twelve minutes, followed by swelling of hands, and in four days, with small cracks at knuckle of index finger of right hand. Black spots on all sides of the fissure. Continual, though gradually increasing ulceration with no tendency to heal. Pain has been excessive, cutting down his sleep to an average of two hours a night; unrelieved by anything except morphia.

Examination of right hand shows characteristic X-ray lesions, few keratoses and many telangiectases. Over the knuckle of index finger is an ulceration with slightly indurated edges, about 3 x 4 centimeters. On the dorsum of the first phalanx, same finger, is another ulceration about the size of the little finger nail. There are several extremely tender points in both ulcerations. Owing to immobilization of the hand, there is some edema and little flexion of the first and second fingers. (See Plate XXXV., Fig. 1.)

Patient was operated upon by me at the Massachusetts General Hospital May 2, 1907. With a wide margin, both ulcers were thoroughly excised, removing all of the skin down to the tendons, and the dorsal aponeurosis, and the skin grafted in the usual manner. Treated with protective for thirty-six hours, and then exposed to air under a cage. The grafts healed soundly, except for one or two small areas at the edge. Patient was discharged from the hospital in ten days. Pain entirely ceased from the time of operation.

Pathological examination showed no definite carcinoma, but in certain spots the epithelium was clearly in a precancerous stage. The papillary layers were depressed below the surface, mitosis was well marked, and there was slight round cell infiltration.

In August, 1907, for the first time, the slight ulceration at the edge of the graft, near the base of the middle finger, was completely healed. There has been no pain, and flexion in the fingers is rapidly returning. By Jan. 1, 1908, there was final and permanent healing; flexion normal; no pain.

March 11, 1908. Fingers could be flexed normally, the graft was movable on the underlying tissues, and appeared to be the most normal skin on the back of the hand. He still suffers from eczema of other parts of the skin.

Summary: Treatment with X-rays for seven years for eczema of right hand. Extremely painful ulceration at base of first and middle fingers.

Average sleep for past six months, without morphia, only two hours. Deep excision; grafting. Complete freedom from pain. Sore at edge of graft unhealed for several months. Present condition: complete healing; normal motion. Precancerous condition, but no definite epithelioma.

CASE VI. — B. S. B.; X-ray manufacturer; personal communication from Dr. M. M. Johnson, of Hartford, Conn. Patient had severe X-ray burn of back of left hand, and over the middle of the sternum. The skin of the back of the hand was thickened, and cut like leather. There were many small nodules and one large open sore, the result of sloughing. A circular incision was made around the open sore, and the metacarpal bone and the tissues were curetted and thoroughly cleaned. A Wolf graft was taken from the arm and stitched in position. At one time there was indication of sloughing at the border of the transplanted tissue, but eventually the result was perfect. The small nodules were excised and but few have returned. The ulcer over the sternum was excised and closed by granulation. Microscopic examination showed chronic inflammatory tissue, without any trace of epithelioma.

CASE VII. — Personal. Dr. Wolbach, Case VI. W. W. G. (physician), of St. Louis; 42 years. Began using the X-ray in his practice in 1897; a static machine. May, 1900, had an itching dermatitis on the dorsal surface of the left hand; this healed in the course of a few weeks. He next noticed, within a short time, trophic changes about the nails, especially that of the index finger. There was alopecia; the skin became dry and reddened. In December, 1900, infection about index finger caused excruciating pain, and in March, 1901, the nail was removed. In 1903 patient was in Berlin, and at that time had numerous telangiectatic areas on the dorsum of hand and fingers, as well as several keratoses. Owing to persistent ulceration about the matrix of the first finger nail, the finger was amputated through the middle phalanx by Professor Sonnenberg. Since then other ulcerations have developed, the most annoying at the base of the middle finger. This remained open until January, 1905, when it was excised by Dr. Bernays, and a Krause graft implanted; the graft healed well. Following this operation he had but little pain but was frequently annoyed by superficial ulcerations about the fingers. For a year or more, prior to December, 1907, he suffered from a slight ulceration near a keratosis at the base of the ring and little fingers. There was also painful ulceration about the matrix of the little finger nail. On Dec. 22, 1907, Dr. Willard Bartlett operated under ether. The middle finger was amputated, through the second phalanx, several ulcerations at the base of ring and little fingers excised and the skin sutured. An attempt was made to extirpate the matrix of the radial half of the little finger nail. At the same time, Thiersch graft was implanted, after excision of a painful fissure over the distal portion of the proximal phalanx of the ring finger; the Thiersch graft was a success. Except for frequent slight injuries to the dorsal surfaces of the fingers, which healed promptly, the hand remained practically free from further ulceration until April, 1908, when an ulcer developed beneath a keratosis near the proximal end of the middle phalanx of the ring finger which, like all the other ulcerations, was exceedingly painful at times. The skin about the thumb remained throughout practically free from lesions, except for a horny growth over the inner surface of the proximal phalanx. There had never been serious trophic disturbances of the thumb nail, but the finger nails, in order of severity, from index to little finger, were profoundly affected. The keratotic spots have invariably been the seat of the ulcerations, excepting that on the amputated index finger which arose from retention of some of the nail-producing membrane.

Patient consulted me Aug. 17, 1908. The left hand is typical in appearance; telangiectases are numerous; there are several thickened papules over the base of the thumb; the ends of two fingers have been amputated; the nail of the ring finger is irregular and thickened; the nail of the little finger has in part been removed, but there has been persistent ulceration on the radial side of the matrix which is extremely sensitive. There is an open ulceration, without indurated margins, over the first interphalangeal joint of the ring finger. There is moderate motion in this joint; the last joint is hyperextended and somewhat stiff. On the backs of the other three fingers are areas of keratoses, scar tissue, and occasional petechiæ. At the base of the cleft, between the thumb and first finger, is an ulcerated papule with somewhat indurated edges.

Operation under ether, Aug. 19, 1908. Several small papules were excised; the wound sutured with horse hair. The ulcerated area on the ring finger was carefully excised with a wide margin down to the dorsal aponeurosis; the edges were bevelled as usual; bleeding was profuse. The ulcerated hemorrhagic and keratotic areas on the backs of the other fingers were also excised, as were several keratoses in other parts of the hand. The entire matrix of the little finger was thoroughly removed and a graft applied. Moderately thick grafts were cut from the arm and sutured in position after the bleeding had ceased. Hand was carefully strapped to a well-padded splint, rubber tissue placed in an imbricated fashion over the grafts. Firm pressure with large gauze dressing applied. The operation lasted about two hours and a quarter.

At the end of thirty-six hours the protective was removed, the hand greased with a mixture of benzoated lard, lanolin, and ichthyol. All the grafts, except one on the forefinger, looked well. August 23, grafts looked fairly well; that on fourth finger improving in appearance. Graft on terminal phalanx of little finger dead. Some sloughing of portions of grafts on first and middle fingers. August 26, large part of graft on forefinger is sloughing, and part of that on the middle. Grafts on the ring and little fingers and dorsum have taken well. August 29, grafted areas thoroughly clean and all portions of unattached skin were removed. September 12, all granulating areas are cicatrizing rapidly from the edges. Discharged on Sept. 15, 1908.

Letter from patient, dated Oct. 22, 1908:

"It is just a few days more than a month since I took leave of you, and since that time my hand has shown continuous improvement. The scabs have fallen from index and middle fingers, and the slight cicatrization about these in no way interferes with the free mobility of the joints. There is a certain elasticity about the skin of these two fingers which I have not hitherto felt. The grafts on the other fingers and at the base of ring finger are simply perfect. Even the slight 'V' shaped defect of graft on ring finger has filled in, so that the entire surface is now uniform. There has been no sign of activity in little finger matrix, and this more than two months since the operation, so that I am not anticipating any further trouble at this point. The dorsal surface of the hand, with the exception of one little point, is entirely free from keratoses, and the places where such were shaved are now smooth and pliable cicatrices; indeed, the appearance of the whole hand shows a vitality which I had not dreamed to be possible."

Pathological report by Dr. S. B. Wolbach. The sections show approximately normal epidermis and corium, except in the middle portion where the epidermis is markedly thickened, the corium infiltrated with lymphoid and plasma cells and without papillæ. There is a marked heaping up of the horny layer of the epidermis, and in one place in the center, where the section is tangential to a hair follicle, there is a downgrowth of the epidermis into the infiltrated corium. These processes from the epidermis are atypical in that the columnar regularly arranged basal layer of cells is absent, and because of included collagen fibrils. The corium just beneath the epidermis does not show the rarefication found in many other sections of X-ray keratoses. The hair follicles and glands are practically normal.

Summary: Marked hypertrophy of epidermis with keratosis and beginning invasion of corium by epithelium.

CASE VIII. — Personal. Wolbach, Case IV. Plate XXXV., Figs. 2 and 3. Miss H., 40 years, is one of extreme interest from many points of view. In brief, the previous history is as follows: seven years ago, patient was operated upon by Dr. Boothby for a so-called fibroid of the uterus, the operation being an abdominal hysterectomy. She was well for a year, when there formed, to the right of the incision, in the abdominal wall, a slowly growing tumor, which was, however, characteristic of a sarcoma, and pronounced inoperable by Dr. Maurice H. Richardson, by whom she was sent to Dr. W. B. Coley of New York for treatment by toxins. He excised a portion of the growth, and found it to be a fibrosarcoma. At first the growth diminished under toxins, but after ten months it was decided that further treatment was not warrantable as the growth was increasing rapidly. The patient was losing flesh, markedly cachectic, very weak, and complained bitterly of pressure symptoms. I am quoting from the report of this case by Dr. Clarence E. Skinner of New Haven, Conn., to whom she was referred by Dr. Bevan of West

Haven, for roentgenization. At this time, he states that the mass covered ten inches from side to side at the level of the anterior superior spines of the ilia, eight inches vertically in the median line, and about five inches antero-posteriorly. Treatment was begun by him on Jan. 28, 1902, and during the next four months she received forty-six applications. The general condition began to improve at once, but the tumor showed little change. Patient went home for a visit, and upon her return it was noted that the tumor had diminished about one-fifth in size. From June 17 to September 3 she received thirty-one roentgenizations. Her general health continued to improve, and the tumor steadily decreased in size, and at the end of this period she resumed her school teaching.

In brief, then, the patient was under treatment for two years and three months, receiving, however, the majority of her applications during the first eight months. The last treatment was given on May 20, 1904, at which time no trace of the tumor was discoverable at examination by Dr. C. A. Bevan of West Haven, and in July by Dr. W. B. Coley of New York. The patient remained perfectly well until August, 1907, though she had noticed that the skin of the hypogastric region was irregularly mottled and much thicker than normal, especially on the right side. Pain, from which she had been free, began to be severe and ulceration commenced. No treatment seemed of avail, the ulcerative process gradually spreading, undermining the skin, which subsequently necrosed. She lost weight and strength, becoming anemic, and suffered severely from the pain. She was referred to me by Dr. Dennett of Winchester, and I first saw her at the Baptist Hospital on March 7, 1908. Examination at this time showed in the hypogastric region more on the right than on the left, an irregular, undermined ulcer about the size of the palm of one's hand, roughly an equilateral triangle, extending to the right side of the pubis, then nearly to the right iliac spine, the other angle approaching the umbilicus. The base of this ulceration is sloughing in parts; in others presenting poorly vascularized granulation tissue, in which necrosis is advancing rapidly. Several areas in the surrounding skin show characteristic appearances. The irregular undermined ulceration with a firmly adherent fibrinous base is surrounded by a very bright scarlet red areola, which bleeds on the slightest touch. Certain points in the ulceration are exquisitely painful on pressure. There is slight fever. After attempting to cleanse wound with antiseptics for a few days on March 16, the whole ulcerated area, with a margin of about an inch, was excised down to the underlying tissue. This excision left apparently normal fat, except over an area two by three inches to the right of the median line, where extremely firm scar tissue marked the site of the original growth. The skin at the periphery, however, was very tough and three times the normal thickness. The whole wound was thoroughly disinfected with carbolic acid, ninety-five per cent alcohol and salt solution, and an attempt was made by tension sutures to diminish the gaping wound. It was dressed with protective tissue, gauze, and swathe. The tissue was given to Dr.

Wolbach of the Harvard Medical School for examination. His report was as follows:

"The specimen consists of a large, irregularly triangular piece of skin and subcutaneous tissue, its 'base' is 9 centimeters, and the sides are 10 and 11.5 centimeters respectively; it is from 2 to 2.5 centimeters in thickness, except at the central portion where it is .5 to .7 centimeter thick. The skin surface shows a large, irregularly triangular ulcer (5.2 x 7.5 x 8 centimeters) conforming in general to the shape of the specimen. The base of the ulcer is depressed from .2 to .6 centimeter below the rough, irregular, ragged, overhanging, and undermined non-indurated edges which are necrotic, reddish gray in color, and bathed in bright yellow purulent exudation.

"The base of the ulcer is bright red to grayish red in color; it is rough and ragged and is irregularly covered with purulent exudate.

"The lower surface of the specimen consists of bright yellow fat tissue through which is a delicate tracery of grayish-white fibrous tissue. In the central part of this surface of the specimen (the thinner area above referred to) there is an area (3.5 x 4 centimeters) of firm, dense, grayish-white glistening elastic fibrous tissue. On section this fibrous 'disc' is seen to be in places in continuity with the base of the ulcer; in other places it is connected by fine and coarse fibrous strands.

"The margin of skin surrounding the ulcer is from 1 to 3 centimeters in width; it is thicker and firmer than normal skin; it is diffusely reddened with a deep punctate erythema, except for numerous, slightly elevated, rounded, whitened areas (.3 to .6 centimeter in size) which have a distinct nodular, almost 'shotty' feel. On section, these areas are .4 to .6 centimeter in thickness; the surrounding skin is .2 to .3 centimeter thick. The bright punctate appearance in the diffuse erythema of the skin surrounding the ulcer is apparently due to marked injection of very small blood vessels.

"Microscopic examination: I. Disc of fibrous tissue, from deeper tissue of lower portion of tissue, forming floor of ulcer.

"This tissue is dense fibrous tissue poor in cells, composed of bundles which run in all directions. There are numerous small arteries and veins and capillaries, all of which are surrounded by narrow zones of lymphoid and plasma cell infiltration. The amount of elastic tissue is small, and that present is in small masses, usually composed of clumps of thick fibers, most abundant in the neighborhood of the larger vessels.

"II. Section of nodules or elevated areas of skin found at the periphery of ulcer.

"The epidermis for the greater part is markedly thin. There are no hair follicles or glands of any sort. The corium is composed of dense fibrous tissue continuous with the deeper tissues so that its normal relations and appearances are gone. There are no papillæ so that the epidermis lies evenly upon dense white fibrous tissue. The corium immediately beneath the epidermis in places stains very poorly. There is marked diminution of collagen fibrils and there are many connective tissue cells of embryonic type. There are many large spaces filled with blood and lined by a single layer of endothelium, which on one side may lie directly in contact with the epidermis. The corium is composed of dense fibrous tissue continuous with the subcutaneous tissue so that there is one compact layer between the skin and fat tissue. This fibrous tissue contains areas with excessive amounts of elastic tissue resembling the masses of elastic tissue found in the stroma of carcinomata. In a few places there is a marked downgrowth of epithelium between dilated vessels. A few areas show branching stalks of epithelium with characteristic pearls or onion bodies. There is marked lymphoid and plasma cell infiltration about the epithelial downgrowths. The atypical character of the epithelium, the presence of pearls and many mitoses and the relationship of epithelium to connective tissue in these downgrowths warrant the diagnosis of epidermoid carcinomata.

"The ulcers: All show similar appearances. The floor is usually covered with fibrin and pus, underlying which is granulation tissue. The epidermis at the edges shows slight downgrowths which do not suggest malignancy. The deep connective tissue contains areas of elastic tissue increase like those described above. Completely and partially obliterated arteries are common in the subcutaneous fibrous tissue. The obliteration is caused by a proliferation of intimal connective tissue. A few nerve trunks are found showing increased connective tissue and compressed nerve fibers. The fat tissue below and adjacent to the ulcers show absorption with organization. The newly-formed tissue contains many large, occasionally multinucleated and grotesquely shaped cells, which are derived from connective tissue as shown by the presence of fibroglia fibrils when stained by Mallory's methods."

The operation was followed by marked reaction, pain, temperature of 102, rapid pulse, exhaustion, and at the end of three or four days continuous sloughing of the edges of the wound, involving the skin more than the fat. The wound at first was dressed with dry gauze, but this was soon replaced, on account of pain, by ichthyol lanolin ointment on bandage cloth. By March 15 necrosis stopped temporarily, and granulation was beginning. The constitutional symptoms were distinctly better, though fever in the afternoon still persisted. On March 20, the condition was as follows: the edges of the skin, in the right groin, had ceased sloughing and granulation was fairly well established. On the left, however, necrosis still proceeded involving only the skin, whereas the fat was granulating healthily. There are new areas of necrosis, varying in size from a dime to a split pea in the thickened skin, near the margin of the wound. There is not much discharge, no enlargement of the glands in either groin, and the general condition of the patient is slowly but steadily improving. Her chief complaint is of pain in the right loin, the lightest contact over the old scar causing extreme shooting pains. This cicatrix is covered with an adherent diphtheritic membrane which is extremely tough. If a portion of it be peeled off, bleeding is free. Cultures were made and sent to Dr. Wolbach, and he reported that bacilli, morphologically similar to diphtheria, were present; inoculation however showed that they were inactive.

April 25: For the past two weeks the wound has been granulating healthily throughout its greater part. The central zone which comprised the old scar is behaving in a most unique fashion. The area, which a month ago was covered by a diphtheritic membrane, is now changed to a smooth, glistening surface, which bleeds on the slightest touch. From the periphery, granulation is slowly stealing in. The skin in the right pubic region is healing soundly, and the epithelium is growing normally over the granulations. Just above the right iliac spine is an irregular dirty fibrin-covered ulcer, about the size of the end of the thumb. The deeper layers of the skin are necrotic. In the central portion, above the umbilicus are a number of similar radiating ulcerations. There are some lymphangitis about the wound. The undermining at the left-hand side of the wound is less marked. From her morning headaches, nervous feelings, and poor pulse, it would seem as if there was continuous, slight infection from the wound, though the temperature is now almost normal. During the summer she lived in a tent, the wound being dressed twice daily. Granulation was slow and epithelium once formed frequently broke down, but over the pubic region, and in the right groin, epidermization continued satisfactorily but slowly and gradually the ulcerations starting in the skin spread, the size of the wound was continually increasing, by their confluence, in all directions. In view, however, of her marked prostration, after her first operation, it seemed better to allow the tissues to slough rather than to remove them. During September her general condition improved very markedly, and with this the central granulating wound took on a more normal appearance. On September 28, I decided upon another operation, at which, under ether, I removed the remaining skin of the anterior abdominal wall, as far out on both sides as the anterior superior spines and upwards to a level of four inches above the umbilicus. At this operation, I only removed the skin, leaving behind as much as possible of the fat tissue. The wound was dressed with protective. Reaction was well marked but was less severe than after the previous operation, and the temperature became normal in ten days.

By Dec. 5, 1908, the patient had gained in general strength and color, and was walking about. Healing has taken place rapidly from the edges, and with the exception of some painful ulcerations in the remaining skin of the left groin the whole wound is granulating for the first time, in a perfectly normal manner. On this date, three long, narrow Thiersch grafts were taken from the arm of a friend, cut into fourteen pieces about three-quarters of an inch square, and scattered over the granulating surface. They were held in position by broad strips of crêpe lisse, covered with protective tissue. After twenty-four hours, salt solution fomentations were applied frequently, and at the end of ten days twelve had healed soundly in position, thus greatly diminishing the denuded area.

Dec. 24, 1908. All of the grafts have grown vigorously during the past week. It seems as if no further grafting would be necessary, as three-fourths of the huge granulating wound is now covered with apparently healthy epithelium. There is in the left groin, outside the edge of the wound, an irregular, unhealthy ulceration, which is extremely sore and tender. This must be grafted at a later period.

Feb. 24, 1909. After three or four weeks all of the grafts taken from her friend were raised by blisters and slowly disappeared. Two weeks ago the left end of the wound was grafted with snips from her thigh; of nine, eight took and have grown well. In view, however, of the slow progress it seemed to me best to operate this morning, February 23. Accordingly, under gas and ether, the wound was thoroughly cleaned with green soap and water, and the granulations vigorously scrubbed with salt solution, followed by a copious saline irrigation. Moderately thin Thiersch grafts were removed from the right thigh, and the whole wound covered. One could not but be surprised by the copious serous discharge from the whole granulating surface. I have never seen anything like it. Whether this was due to some peculiar condition, or possibly to the irritation of the green soap, I do not know. The grafts were covered with protective, gauze was then placed carefully over the uneven surfaces, and held in place by adhesive plaster. The thigh was covered with protective and bandage. A snug swathe was then evenly fitted over the whole abdomen, making all possible pressure.

April 1, 1909. With a few exceptions, all of the Thiersch grafts took, and, with their continued growth, at present the huge wound is continuously covered with sound, thick, and somewhat movable skin, except for an area on the right hand upper margin, 2 x 3 centimeters. There is no tenderness in the scar, and no pain. Patient has been up and about for three weeks, having been in the hospital thirteen months. Owing to continued flexion of the thighs there is some subluxation of both knee joints, and she walks clumsily and somewhat in a stooping manner, owing to the contraction of the anterior muscles of the body. There is no evidence of recurrence of the sarcoma. Her general condition is excellent.

Summary: This patient, after unsuccessful treatment of ten months with Coley's serum for a fibrosarcoma of the abdominal wall, was entirely cured of this condition by one hundred and thirty-six X-ray treatments, covering a period of two years and three months. She was well for four years, except for some thickening of the skin and telangiectases, when severer and characteristic X-ray lesions developed, not only in the scar of the shrunken tumor, but in the skin over the whole anterior abdomen. In addition to the ulcerations of the skin, which not only refused to heal under all treatment, but steadily increased in depth and area, there were numerous papules of undoubted epidermoid cancer. At the first operation the ulcerated areas of the lower abdomen were thoroughly excised with wide margins, down to the scar of the tumor and the anterior aponeuroses. The operation was followed by marked infection, which was questionably diphtheritic. The wound was extremely sluggish and granulations very slowly formed, covered by unhealthy fibrin. General condition poor. After months of convalescence, granulation gradually established, and epidermization slowly took place from the pubic and inguinal regions, but the thickened skin ulcerated upward and laterally, with great pain and some fever. Six months after the first operation I decided under ether to remove the remaining thickened skin of the anterior abdominal wall for a distance of four inches above the umbilicus, and laterally to a level with the anterior superior spines. The skin and upper half of the subcutaneous fat alone was removed. The wound was dressed with protective. After two months, the wound at that time granulating healthily, a number of Thiersch grafts were removed from the arm of a friend and applied over the large surface. In three weeks, healing was almost complete, but in two more the apparently healthy grafts began to blister and come away. In three weeks more, patient was etherized and grafts from her own thigh placed in position. These at the present time, thirteen months after the first operation, have grown soundly and the whole wound is healed. This case should serve as a warning not to use Thiersch grafts from other people, unless the condition of the patient contraindicates ether.

CASE IX. — M. K. K. (physician); 40 years; X-ray operator, Philadelphia. Began work in 1899. In 1901, hands showed usual early lesions; telangiectases, ribbing of the nails and thickened fissured skin. In 1903 the superficial ulcerations and keratoses shown in the photographs, involving the lower parts of the hands and fingers. By January, 1908, there was general improvement of the dorsum of the hands and fingers of the right hand, but an extensive and suspicious keratosis at the base of the fourth finger. For two years there has been ulceration of the two terminal phalanges of the middle finger of the left hand, and for eighteen months a similar condition over the first phalanx and distal joint of the fourth finger. By December, 1908, the large keratosis on the right hand, under treatment, has almost disappeared, but the ulcerations on the end of the middle finger of the left hand were typically malignant. The ulcerations and a keratosis on the ring finger became infected, and an exuberant growth appeared increasing rapidly in size. On April 4, 1909, the middle finger was amputated at the end of the first phalanx, and the ring finger at the knuckle. There were no glands in the axilla. (Pathological diagnosis: epidermoid carcinoma; Plate XXXV., Figs. 4, 5, 6.)

CASE X. — M. N.; 35 years; Swede. Personal communication from Dr. A. D. Bevan of Chicago. In April, 1902, had abscess of obscure nature in left lower quadrant of abdomen. This was opened and found to be fecal and drained. A fecal fistua resulted, which was closed at operation August, 1902. He returned again October, 1902, with symptoms of intestinal obstruction and an exploratory laparotomy was performed and a tumor-like mass found in the descending colon. The operation was purely exploratory. December, 1902, he again had symptoms of obstruction; another operation was performed; some adhesions between the small intestine and descending colon separated, and a Meckel's diverticulum was found and removed. This apparently had nothing to do with his illness. The tumor-like mass in the colon was of uncertain diagnosis; we regarded it as either carcinoma, syphilis, or tuberculosis. He was given antispecific treatment and was given a large number of X-ray exposures over the mass. This resulted in rather an extensive X-ray burn, which healed slowly. The swelling in the abdomen disappeared and the man made an apparently complete recovery.

October, 1908, he returned to the hospital with several irregular ulcers in the scar of the X-ray burn. It was thought that they were suspicious of carcinoma and a large area about 6 x 6 inches containing the scar of the old X-ray burn and these ulcers were dissected off, and the surface covered with skin grafts. The microscopical sections from the margin of the ulcers show a suspicion of beginning carcinoma. There is proliferation of the epithelial cells with processes extending a moderate depth in the subcutaneous tissue, in several portions of the section, but only at these points is the basement membrane broken. There is some round cell infiltration; nowhere is there a separate island of epithelial cells or epithelial pearls. From the microscopical section I am inclined to regard it as a beginning epithelioma.

Summary: Large number of X-ray exposures for abdominal tumor; extensive burn which healed slowly. Latent period at least five years, when several irregular ulcers developed in the scar. A large area 6 x 6 inches dissected off with successful skin grafting. Pathological report: beginning epithelioma.

Case XI. — Personal. E. W. C.; aged 37 years; admitted to the Massachusetts General Hospital Sept. 3, 1907, with the following history: Shortly after beginning work with the X-rays, ten years ago, patient noticed desquamation and ulcerative areas appearing on the dorsum and knuckles of left hand. He also developed telangiectases of left thigh and lower abdomen. Although he has long since ceased to expose himself, the parts have continued to ulcerate, until some of them have taken on a more deep-seated epitheliomatous character. He has tried all sorts of washes and ointments, exposure to sunlight, arc light, with and without screens, and the so-called luecodescent light. In his case, all of these agents were harmful; the relief reported by some, he explained, as due entirely to the heating effect, for the pain, which prevented sleep, would often yield to treatment for fifteen or twenty minutes, with an electric heating pad.

Examination of left hand shows the usual telangiectases, atrophy of the skin, with numerous keratoses and papules, and several ulcerated areas; two in particular appearing most suspicious — one at the base of the first finger, and another in the cleft between it and the thumb. There were superficial ulcerations with keratoses over the interphalangeal knuckles of the first two fingers, and another rather extensive keratosis at the base of the ring finger.

Under ether, Sept. 4, 1907, all of these areas were excised, the suspicious ones down to the subcutaneous tissues; the others well into the corium. Skin grafts of moderate thickness were applied to the wound, after bleeding had ceased. Protective tissue, just outside the periphery of the grafts, with firm pressure applied by a gauze roller, the hand having previously been immobilized upon a well-padded splint. On the 5th, rubber tissue removed, and grafts exposed to air, covered with ointment. On the 7th, grafts looked finely. On the 8th, grafts were all looking well, except one over the fourth metacarpo-phalangeal joint, which is a little white in the center, where a small necrotic area has developed. On the 9th, all grafts had taken, except the one previously mentioned over the knuckle of the ring finger. Discharged on September 12, eight days after operation.

Pathological examination showed that ulceration at the base of the forefinger was undoubtedly epithelioma. In two other places, a precancerous condition was present; the tissues from other parts showed superficial ulcerations or keratoses.

Quotation from letter dated Sept. 15, 1908 : "For your records, I have to report that all the grafts grew successfully, and most of them have contracted much more than I expected. They are all in splendid condition, except that there is a tendency to the formation of keratoses at some of the margins. It seems to me that this condition, so common in all X-ray hands. nearly always starts from a small abrasion, and that the trauma at the margin of the incision for the skin graft is just the sort of injury which has produced this kind of growth. I have been able to improve the condition very much by having these spots treated with liquid air, after which there is no tendency toward recurrence. There are no signs of metastatic involvement of the epitrochlear or axillary glands."

Summary: Characteristic X-ray hands. For several years, intermittent healing and ulceration; finally persistent ulceration in spite of treatment by various forms of light, in addition to the usual treatment. Eight excisions; all grafts successful. One undoubted carcinoma; two precancerous lesions. All keratoses which formed at margins of graft successfully treated with liquid air. No return of ulcerations or of disease. Satisfactory result.

CASE XII. — S. L. (physician), New York. Personal communication in regard to his own case. "Frequent excisions of epitheliomatous areas, which broke down from time to time, has reduced them to a definite number: five on my left hand of very small size. Of course I have absolutely given up the use of the X-ray, and have even had my machine removed from the office. I still find that the left hand resists chemicals very badly. Too strong or too frequent use of bichlorid will give me an eczematous condition, which immediately lights up the spots from which the epitheliomata have been removed."

CASE XIII. — C. L. L. (physician). Several years ago developed an epithelioma of his forefinger, and fearing excision or even amputation, lest metastases result, applied the X-ray very vigorously, with the intention of destroying all malignant cells before operation. He produced a slough which extended to the bone, after which the finger was amputated and healed by primary union. There are a few suspicious areas over the fingers of the same hand; no glandular involvement.

Case XIV. — Mr. S., New York, radiographer. Amputation of one finger for epithelioma developing in an X-ray lesion. Now well.

Case XV. — Personal. Wolbach, Case V. Plate XXXVI., Figs. 7 to 11 (inclusive). F. H. S. Patient sent to me by Dr. Percy Brown. First used X-ray apparatus in the spring of 1897; static machine. Received acute dermatitis on back of left hand, which after healing showed characteristic pigmentation. He has had a few attacks of acute processes since then, but not until three years ago did an ulcer form on the back of the left hand between the knuckles of the first and second fingers. This healed up several times and broke down again. Over the backs of the hands in various places keratoses have formed which became black and then spontaneously fell off. Sometimes has scraped them off with pumice stone. Except for the inconvenience of dressing the ulceration he has had practically no pain in his hand since the original dermatitis. For many years he has noticed that the skin on the front of his chest has become thickened; here and there are somewhat more indurated spots; the whole chest is covered with the characteristic venules. For a number of months he has noticed that the pores of the tip of his nose have filled up, occasionally discharging sebaceous matter. For two months and a half he has noticed a warty growth on tip of nose about the size of a pea.

Examination: On the tip of the nose is a warty growth on which a scab has formed of the size before mentioned. This discharges some serum, no pus; is not sore. The chest presents the characteristic appearance of chronic X-ray dermatitis with telangiectases and some pigmentation. There is thickening of the skin in plaques, but no ulceration. The skin of the back of the left hand is thickened in some places, especially over the joints where it is atrophic and cracked. As shown in the photograph, there are about half a dozen keratoses, the skin about them being thickened to twice normal. At the cleft between the first and second fingers is a well-marked induration with a keratosis growing on the distal part. Above this are three open ulcerations, the distal one having slightly raised indurated edges. The proximal one is flat, non-indurated, appearing like smooth granulation tissue. Diagnosis of the distal ulceration probably beginning epithelioma; of the proximal one, simple ulcer.

Operation under ether March 29, 1908. Ulcerated area on back of hand carefully excised with broad margin down to underlying veins, fat and tendon of middle finger. Edge of thickened skin bevelled. Several indurated areas and keratoses were either excised or shaved down to the deep skin. All of the raw places were skin grafted from the left arm. The growth on the tip of the nose was shaved off deeply. All of the wounds were covered with protective and the hand firmly bandaged on a splint and elevated. No dressing applied to nose.

For purposes of microscopic examination, an indurated superficial ulcerated papule in the cardiac area of the chest was removed by an elliptical incision, and the skin approximated with silkworm gut sutures.

Convalescence: the usual protective dressing remained in position for twenty-four hours, then ointment and cage. Patient discharged in a week.

Pathological examination by Dr. Wolbach.

1. Keratosis from nose. (Plate XXXVI., Fig. 11.)

The epidermis is greatly thickened and irregular and the horny layer is heaped up, forming a thick layer of keratinized cells. The cells of the stratum germinativum are large, vesicular, and present mitoses. In some by high power fields as many as ten mitoses are found. The processes extending into the corium for the most part present an orderly arranged basal layer except in a few places where the epidermis is infiltrated with lymphoid and plasma cells. The corium immediately beneath the epidermis is thinned in places. Some of the smaller vessels are filled with polynuclear leucocytes. There is everywhere extensive infiltration with lymphoid and plasma cells beneath the epidermis. The epidermis on either side of the keratosis is practically normal.

The most striking feature of this tissue is the extraordinary number of mitotic cells in the epithelial processes and the change in the character of the cells from the compact, basic-staining appearance of the normal epidermal cells to the large vesicular neutral-staining cells found in the processes beneath the keratosis.

2. Large ulcer from hand. (Plate XXXVI., Fig. 8.)

The ulcerated surface is practically the exposed surface of a vascular new growth of epithelium, atypical in type and containing many whorls of keratinized cells. This tissue is microscopically a typical epidermoid carcinoma and there is definite evidence of invasion of the corium. The epidermis on each side of the central mass is irregularly thickened in places and thinned in others. The corium immediately beneath the epidermis is rarefied. A few arteries show thickened walls. No sweat glands or hair follicles found in the sections.

3. Keratosis of hand between index and middle fingers.

The same general picture is found here as in the tissue removed from the nose. The rarefication of the corium beneath the surrounding approximately normal epidermis is more striking than in the case of the nose. There is less infiltration beneath the keratosis, and the epithelium shows comparatively few mitoses. No positive evidence of malignancy.

4. Tissue from the chest. (Plate XXXVI., Fig. 10.)

Consists of a semicircular piece of tissue the convex border of which is covered by epidermis. The central portion of the tissue is very similar to that from the ulcer of the hand in that we have a new growth of epidermis supplied with many blood vessels and having no definite epidermal covering. The projecting stalks of the central mass contain many whorls of keratinized cells, and the relationship to the connective tissue of the corium leaves no doubt that the tissue is that of a new growth, epidermoid carcinoma in type. The corium beneath the surrounding epidermis shows the same rarefication noted in other cases.

General Note: A description of the new growths from the hand and from the chest hardly seems necessary. In both locations the picture is that of typical epidermoid carcinoma. Mitoses are numerous and the masses of epithelium enclose remnants of elastic fibers and collagenous connective tissue. In the corium, immediately beneath the new growth, there is more increase in elastic tissue, otherwise the changes in the deeper tissue are similar to those noted in the cases of H. and S.

April 22, 1908. Patient visited me the day before yesterday. All of the skin grafts have healed and now show the usual increase in vascularity and are raised above the surrounding skin. The large graft on the back of the hand shows a slightly hypertrophic condition, but is beginning to be movable on the underlying tissues. Several areas of keratoses are slowly reforming.

May 30, 1908. The pathological report that the papule excised from the chest was carcinoma came as a complete surprise. This fact was explained to the patient, and on May 29, under cocaine, the scar of the former operation was excised with a wide margin down to the subcutaneous fat; wound closed with sutures.

December, 1908. Present condition: the nose is slightly flattened at the tip and shows a slight scar. Grafted area upon the hand shows no evidence of recurrence of the carcinoma, and is quite freely movable upon the underlying structures. There are several cracks and fissures, as well as keratoses on the various parts of the hand, but nothing suggesting epithelioma. There is no return of the cancer at the site of the excision over the breast.

Summary: Early acute dermatitis in 1897; innumerable exposures of chest for fluoroscopic examination of heart. Characteristic lesions of chest and left hand; ulceration of hand persisted for three years; no pain. Operation: excision of keratosis on tip of nose, of ulceration of hand and keratoses; successful skin graft. Excision of papule on chest for examination, which proved to be undoubted epithelioma. Prognosis doubtful, as the remains of the indurated tissues of chest may develop carcinoma. Very satisfactory graft.

CASE XVI. — G. W. D.; 32 years; radiographer and salesman. Patient of Dr. F. E. Pinkham, Providence, R.I. Patient began work in 1896. Had amputation of ring finger of left hand at the Worcester City Hospital in 1905, on account of ulceration and exuberant growth which had been microscopically examined and pronounced to be an epithelioma. He entered the Massachusetts General Hospital April 7, 1908, having noticed, six weeks ago, a hard lump in the axilla which, though slightly tender, was not painful. In the past three weeks there had been rapid growth of this tumor. No loss of weight.

Examination of left hand: ring finger amputated at the metacarpo phalangeal joint. On the dorsum of back of hand are several small keratotic areas which have been present since 1899. Skin of dorsum of hand is hard and indurated. No enlargement of left epitrochlear gland. In the left axilla is a tumor about the size of a hen's egg, which is slightly elastic and tender, and more or less fixed to the surrounding tissues. No palpable glands above clavicle.

Operation, April 7, 1908. Incision made through center of axilla over the mass, parallel to the lower border of the pectoralis major muscle. Skin dissected back; axillary fascia opened; gland found to be very hard, possibly slightly fluctuating in the center. Incision made into it, specimen given to Drs. Kidner and Wright for examination. Frozen section showed undoubted epithelioma. The center of the gland was softened and about a teaspoonful of pus or epithelial débris escaped. An incision was then made upward from the other, at right angles to the collar bone. The pectoralis major muscle was divided and retracted, and the pectoralis minor divided at its insertion into the coracoid process. By blunt dissection the fascia covering all of the muscles forming the axillary triangle was removed with the inner portion of the pectoralis major, minor, and axillary third of the latissimus dorsi, and some of the subscapularis; bleeding was quite profuse. The long thoracic nerve was preserved, but some of the branches of the subscapular and the latissimus dorsi were cut. A part of the axillary vein in contact with the gland was resected for a distance of two inches, but the collateral branches of the brachial veins, entering above, carried on the circulation adequately. With the arm flexed and brought forward, the nerves, axillary artery and vein were held forward with retractors and with great care, the whole mass of gland-bearing fat was removed well above the clavicle. It was obvious that in order to get good motion in the arm, a plastic would be necessary. The sternal portion of the pectoralis major muscle was therefore freed from its attachment, and brought downward over the axillary vessels and sutured to the insertion of the muscle. A "U" shaped flap of skin was left attached to it, and utilized to close in the axillary opening.

Convalescence: Some infection occurred from the pus in the broken-down gland, accompanied by fever for several days. Several stitches were removed and a portion of the pectoral muscle sloughed, but patient was discharged with a granulating wound May 4, twenty-five days after operation.

Letter dated Dec. 7, 1908: "I thank you very much for your enquiry, and am greatly pleased to report that I am enjoying excellent health. After leaving the hospital, the wound granulated very nicely and closed up beautifully within three weeks. I have now a fairly good use of the arm."

Summary: X-ray work begun in 1896; ring finger amputated in 1905 for an epithelioma of over a year's duration. Patient well until February

1908 (three years) when malignant disease appeared in the axillary glands; epitrochlear not involved. Very radical dissection of axilla; some infection of wound. Sound healing in six weeks; good use of arm. At present well.

Pathological report by Dr. Wolbach. "Microscopic description: methylene blue and eosin; Mallory's connective tissue stain, phosphotungstic-hematein stain. Paraffine sections. Tissue consists of large lymph node infiltrated with new growth consisting of anastomosing stalks of epithelial cells containing many whorls of cornified cells. The whole is supported by a fairly dense connective tissue reticulum. The picture is that of typical rapidly-growing epidermoid carcinoma. Mitoses are extremely numerous."

CASE XVII. — Personal. (Plate XXXVIII., Figs. 22 and 23.) H. G.; 48 years. Consulted me first in January, 1908. He had been working since the very beginning in the manufacture of X-ray tubes. The first indication of any trouble appeared in the shape of a red, itching rash, of which he took very little notice, supposing it to be caused by acid fumes. This condition was succeeded, the latter part of 1898, by the appearance of a number of callosities on the back of the hand, which about the spring of 1899 had changed into warty growths, which he tried to remove by pulling them off. The removal was attended by severe pain, and free bleeding, and seemed to cause them to increase in size and multiply. These warts and other scaly areas were removed frequently by means of a live cautery, only to reappear in increased size. His physician used almost every known salve but the only result was to allay the pain somewhat. "No pen can describe the sensitiveness or the pain of these burns." "One peculiarity of my case is that from the frequent examination of my body with the fluoroscope by hundreds of doctors, the skin of my chest became black and hard, like parchment, giving me, however, no inconvenience and never becoming sore; in less than a year, this blackened skin all came off in one piece, leaving the chest clean, without a scar, but of course removing all hair." For seven years, then, there has been ulceration, induration, and keratosis over an area about 3 x 1½ inches on the back of the left hand. This had been treated in various ways, and during the past six months has shown signs of improvement, under a dressing of vaseline, turpentine, and creosote. The backs of both hands show many keratoses, but the usual telangiectases are remarkable for their absence. On the back of both wrists is an area about the size of a pea, which is hard, not sore, ulcerated in the center, with indurated edges, presenting the characteristic appearance of an epithelioma. A similar growth started three months ago on the left forehead. This soon acquired raised and everted edges and was operated upon by a surgeon in Hartford, but in a month had recurred, and was larger than before. It was again excised, but rapidly returned. On examination of the forehead there appeared a growth about the size of a quarter, gangrenous in the center, with palisaded edges. There was induration about it, and the growth was firmly fixed at the center to the underlying tissues. No glands could be made out in the parotid region or at the angles of the jaw.

Operation Jan. 17, 1908; ether. After thorough disinfection with pure carbolic acid, the growth on the forehead was excised with an ample margin, removing a circle of tissue about two inches in diameter. The incision was carried down to the periosteum, which was removed with the growth. Section of the tumor showed that it involved the periosteum. On this account, the outer table was chiselled away for an area the size of a half dollar, and the inner table was also removed, over an area the size of a quarter, exposing the dura and the mucous membrane lining the frontal sinus. A complicated plastic operation was done, and the wound closed without drainage. The two epitheliomata over the wrists were excised and the skin sutured. Next, with considerable margin, the ulcerated and thickened skin over the back of the hand was removed down to the veins, subcutaneous tissue, and tendons. A rather thick Thiersch graft was applied and sutured in position with firm pressure. The graft grew by primary union, except for a small area at the base of the little finger, which healed within a few weeks. Patient discharged at the end of ten days.

The two discrete epitheliomata from the wrist were lost. The microscopical examination from the skin of the back of the hand by Dr. J. H. Wright was as follows: The surface of the portion of skin removed is covered with crusts, scabs, and discrete and confluent wart-like elevations. After fixation in Zenker's fluid, paraffine sections were prepared from six different places. The lesions found in these may be summarized as follows: "The stratum corneum is very much thicker than normal, and shows cyst-like cavities of varying size containing hyaline material. The stratum germinativum in places is either thicker or thinner than normal, and the interpapillary processes are irregularly variable in size and shape or are wanting. In a few places the cells of this stratum show atrophy with exaggeration of the intercellular spaces and there is infiltration with leucocytes. The cells of this stratum also show some imperfect cornification. In one section two circular masses of cornified epithelium, like the "pearls" of epidermoid carcinoma, are present in interpapillary processes. In another section over a small area, the stratum germinativum is prolonged into small columns of atypical epithelial cells which lie in the superficial portions of the corium. In the corium about the cell masses is some infiltration by cells of the lymphoid series. I am inclined to interpret these appearances as indicating an incipient carcinoma at this point. The corium generally shows an increase of its connective tissue over the normal, and this connective tissue in places is in the condition of hyaline degeneration. The papillæ vary considerably in size and in places contain greatly dilated capillaries. Some papillæ are in a condition of myxomatous degeneration and some others show more or less transformation of the connective tissue into hyaline material. In places, small areas of the corium are infiltrated with cells of the lymphoid series.

Examination of the recurrent tumor on the forehead brings out a difference of opinion between Dr. Wolbach and Dr. Wright.

(See Plate XXXVIII., Fig. 22.) Pathological report by Dr. Wolbach. Tissue from forehead. The sections consist of tumor tissue with adjoining necrotic tissue. No normal tissue in sections, except for a narrow strip of dense fibrous tissue on one edge of a few sections. The microscopic picture is that of invading cells or of cells having proliferated in situ, distending the meshes of connective tissue. There are bands of dense collagen fibrils separated by large, irregularly shaped cells, many in stages of mitosis. That the connective tissue present is not wholly the product of the tumor is shown by the presence of normal nerve bundles. Between the tumor cells and between small groups of cells there is in places only a small amount of collagen fibrils (Mallory connective tissue stain). The tumor cells are very irregular in shape and in size. Many are multinucleated. There is no definite arrangement of cells and in places the appearance suggests that these cells may have arisen in situ by multiplication of preëxisting cells. In other places, the picture is definitely that of a tumor. The nuclei are large, and each contains a large nucleolus. Where numerous nuclei exist in a single large cell, the nucleoli are unusually conspicuous in each nucleus. Mitoses are abundant in all portions of the sections. Multipolar mitoses are common. The smaller tumor cells are round, oval, and spindle-shaped. The larger very irregular in outline and usually have many slender processes. A few tumor cells show delicate fibrils (Mallory fibroglia stain) running parallel to the long diameter. For this reason and because of the arrangement of the tumor and the apparent presence of intercellular collagen fibrils the tumor should be classed as a sarcoma of connective tissue origin.

Pathological report by Dr. J. H. Wright: "In accordance with your request I confirm my verbal report to you made some time ago upon my views as to the nature of the tumor of the forehead in the case of Mr. G. Although the histological character of this tumor is very suggestive of sarcoma, I believe that the essential cells are of epidermal origin, and that the tumor should be regarded as carcinoma originating from the epithelium of the skin of the forehead. I am led to this belief for the following reasons: the situation of the tumor, the presence of fibrillation in some of the cells reminiscent of the fibrils of the cells of the epidermis, and the existence at the periphery of some of the cells of structures strongly resembling the characteristic 'prickles' of the epidermal cells."

Quotation from letter, March 6, 1908: "My hand has healed in good shape and most of the thickened spots on my fingers are disappearing. I can about half shut my hand. My forehead is all right except for some tightness of the skin and absence of normal sensation. The spot, where the growth had been, is tight and does not move."

Examination, Aug. 5, 1908. Sensation has been gradually coming back to forehead, which he can wrinkle almost normally. There is very slight thickening on the inner side of right eyebrow where the flap was reflected. The scars are hardly visible. There is no induration or pain suggesting any recurrence of the disease. The back of the left hand is entirely healed; the graft is movable over the underlying structures. The backs of the fingers still show keratoses, which he is cutting and paring off. He can flex and extend fingers normally. In every respect a most excellent result.

Summary: Intermittent ulceration of back of left hand for seven years, improving for the past six months; excision down to aponeuroses and tendons; successful skin grafting. Microscopic examination showed commencing epithelioma. The longest case of persistent ulceration, without cancer, in the series. Thrice recurrent growth of forehead, which involved the periosteum; removal of bone down to dura and frontal sinus; complicated plastic operation. Opinions differ as to the nature of the growth; Dr. Wolbach classifying it as a sarcoma; Dr. Wright believes it to be a carcinoma. One year after operation, no evidence of recurrence.

CASE XVIII. — Personal. (Plate XXXVII., Figs. 13, 14, and 15.) J. G.; 34 years; X-ray operator. Patient has suffered for several years from the milder forms of X-ray lesions, atrophy of the sweat glands, eczema, ribbing of the finger nails, telangiectases and keratoses. During the winter he has a great deal of pain and trouble from fissures over the extensor surfaces of the joints. In May, 1906, numerous excoriations and fissures were excised and successfully grafted. On Sept. 15, 1906, there was a recurrence of ulceration near the radial border of the terminal phalanx of the ring finger. On October 18 this ulceration was freely excised, and the radial fourth of the nail with its matrix extirpated. The skin at the margin of the nail was approximated with plaster and another graft applied; sound healing in ten days.

Pathological report by Prof. F. B. Mallory of the Harvard Medical School: "The section shows a small mass of actively proliferating connective tissue cells, covered with epidermis, which is edematous and contains fairly numerous mitotic figures. At one or two points the epidermis is lacking, and a little granulation tissue has formed. In these areas, and beneath the epidermis, is a small amount of infiltration with cells (chiefly lymphocytes) of inflammatory origin. The proliferating connective tissue cells occur diffusely scattered among the bundles of collagen fibrils of the corium. Mitotic cells are numerous and often multiple. Many of the resulting cells are large and often contain large lobulated or multiple nuclei. The lesion is an active, in some ways atypical, proliferation of connective tissue cells."

The finger remained healed until the middle of March, 1907, when the distal part of the graft on the radial side began to increase in size and show well marked vascularity. After two weeks there was a slight discharge near the edge of the nail. By April 23, 1907, the growth had become decidedly larger and was apparently extending backward into the proximal graft. The terminal joint was accordingly amputated under gas and ether; primary union.

Pathological report by Prof. F. B. Mallory of the Harvard Medical

School (see Plate XXXVII., Fig. 15): "The section shows an oval, cellular mass of tissue, partially surrounded by more or less normal fibrous tissue. The cellular mass at its outer end is ulcerated and covered with fibrin, cells, and dried necrotic tissue. The more normal tissue is covered with epidermis. The oval cellular mass of tissue is quite sharply defined and is limited by a layer of dense, fibrous tissue. It is composed of rapidly-growing connective tissue cells and of a small number of thin-walled blood vessels. The connective tissue cells are typical, that is, they have flat, oval nuclei, and contain one to three coarse chromatin masses. The cytoplasm is made out with difficulty. In places the cells are bordered by very delicate fibroglia fibrils. Everywhere the cells are separated from each other by a relatively large amount of ordinary (collagenous) fibrils. The cells and their fibrils tend to form small bundles which run in all directions. Mitotic figures are numerous, one to three showing in nearly every oil immersion field. While the cellular mass of tissue at its base is sharply defined, on both sides, it gradually blends with the adjoining connective tissue. The adjoining tissue of the finger shows infiltration with numerous groups of lymphocytes. Otherwise there appears to be no change in it. It is difficult to give a positive diagnosis in this case. The rapidly-growing mass of connective tissue may be either an unusual form of reparative action on the part of connective tissue or it may be a connective tissue new growth, namely, a rather slow-growing spindle cell sarcoma. Personally I favor the former view, owing to the lack of any definite evidence as yet of invasion."

The finger remained well until August, 1908, when on the ulnar side of the same there appeared an induration in the skin which was neither tender nor painful, but gradually grew until it was the size of an old-fashioned three-cent piece. There was no ulceration, the tumor had slight vascularity and was movable to some extent upon the underlying structures. In view of the doubt as to the true pathology of the recurrent growths on this finger, amputation at the interphalangeal joint was performed in November, 1908; primary union.

Pathological report by Prof. F. B. Mallory of Harvard Medical School: "This lesion, like No. 2, is quite definitely outlined. In places at the periphery, however, the cells extend among the bundles of old collagen fibrils of the corium. The tissue is fairly cellular and contains mitotic figures, but they are not so numerous as in lesions 1 and 2, and are rarely atypical. The cells and fibrils are separated by a small amount of fluid (edema). The sections from these lesions show masses of rapidly-proliferating connective tissue cells. In numbers 1 and 2, especially, the cells are often atypical. The mitoses are frequently multiple and large multinucleated cells result from them. All these lesions suggest the possibility of being fibrosarcomata. It seems wisest for the present, however, to regard them as more or less atypical growths of connective tissue cells under conditions not fully understood. If later it should be proved that X-rays cause similar connective tissue growths which give rise to metastases or to evident invasion of other tissues (true fibrosarcomata), these lesions might be regarded as of a similar nature, but at present such an assumption is not justifiable."

Pathological report by Dr. Wolbach. Amputation of middle phalangeal joint of ring finger, left hand. Beneath the skin of the distal portion on the ulnar side of dorsal surface is a firm, resilient, yellowish, ovoid nodule, .8 x .6 centimeter in diameter. This nodule is completely covered by epidermis and is not attached to the bone or tendon.

Microscopic examination: The nodule is composed of loose cellular connective tissue, the cells of which form bundles and whorls running in all directions. The amount of collagenous intercellular substance is much greater than that in the first specimen removed and examined by Dr. Mallory. The collagenous fibrils are more widely separated than in normal connective tissue, suggesting a slight amount of edema. The cells resemble those of normal connective tissue. The nodule is fairly sharply demarcated from the immediately surrounding practically normal connective tissue of the corium by means of the concentric flattened arrangement of the cells and fibrils of the latter. There is no definite capsule. There is no evidence of invasion. There are numerous mitotic figures throughout the nodule, all of them normal in type. The tissue is supplied with numerous small, thin-walled vessels and capillaries, uniformly distributed. The epidermis covering the nodule consists of a smooth thin layer lying directly upon the tissue described and which presents an unbroken convex surface. The horny layer of this covering epidermis is thin. The stratum germinativum is poorly differentiated; it consists mainly of slightly swollen pale-staining prinkle cells of fairly uniform size. The deepest layer is regularly apposed to the underlying tissue in the manner of normal epidermis. Where the nodule adjoins the normal tissue there is a deep downgrowth of epidermis in the form of slender stalks partially embracing the new tissue. The surrounding epidermis is slightly hypertrophic and shows slight irrelevant changes. A positive diagnosis is not possible in this case, as the same discussion used by Dr. Mallory in his description of the tissue removed applies here. Against the view that the tissue is granulation tissue is the lack of signs of active inflammation, the presence of unbroken epidermis and the absence of a vascular arrangement. Favoring the view that we are dealing with a slowly-growing connective tissue tumor (fibrosarcoma) is the presence of numerous mitoses, and against this view is the lack of evidence of invasion.

Summary: Mild chronic X-ray lesions, numerous skin grafts for keratoses and fissures over knuckles. Primary union. Recurrent growth of the matrix of ring finger nail, left hand; amputation. Occurrence of a fibrous tumor on the ulnar side of stump one year afterwards; amputation through first phalanx. Pathologists differ as to the character of this growth, some believing it to be an unusual form of granulation tissue; others classifying it as a sarcoma.

CASE XIX. — Personal. (Plate XXXVII., Figs. 16, 17, 18.) W. J. D. (physician); 32 years. X-ray operator. Began work with a large static machine in March, 1896; in October of same year a powerful twelve-inch coil was used. In November, 1896, the first severe dermatitis took place. This subsided under treatment, but was followed in April, 1897, by an extremely severe general dermatitis with pain beyond description. All kinds of washes, ointments, and powders were used with orthoform to relieve the pain. My first graft was applied to an ulcer on the tip of the left forefinger on July 10, 1897. On Aug. 13, 1897, fourteen different grafts were applied after excision of as many ulcerated areas. The extreme pain ceased at once and the great majority of the grafts healed soundly. Between this date and 1902 there were seven other similar operations under ether. Most of the grafts were successful, but in spite of several attempts failure was constant over ulcerations on the ends of the ring finger of both hands.

In July, 1902, another attempt at excision and grafting was made; it failed. In October, extremely painful, angry looking ulcers with indurated edges had formed upon the ends of both ring fingers, these were amputated. The report from the pathologist was unmistakable carcinoma. In consequence, on Oct. 31, 1902, both of the ring fingers were amputated at the knuckles. From October, 1902, until June, 1905, a dozen or more operations consisting of partial amputations, excision of growing ulcerations and keratoses were performed. In May, 1905, for the first time in eight years, the patient was free from pain and no dressings had to be worn. In June, the left hand was practically well, but keratoses broke down on the base of the middle finger of the right hand, and there was some ulceration near the matrix of the thumb nail. In November, 1905, an undoubted epithelioma was excised from the base of the third finger of the right hand, and numerous other ulcerations were skin grafted; some of these showed a precancerous stage.

By April, 25, 1907, after ten years of treatment, and twenty-five operations under ether, the condition of the hands was as follows: Left hand: amputation of little finger through the interphalangeal joint; amputation of ring and middle fingers at knuckles; fourth finger shows slight ulceration at its base, and a small ulcer in the middle of the back of the hand; there are numerous keratoses, but no ulcerations. Right hand: the thumb is useful, but its ulnar side is covered with thickened epithelium at the base of which is a small ulceration. For two months there has been ulceration over the joint between the first and second phalanx of the forefinger, with flexion of this joint; this ulceration strongly suggests malignant disease. The middle finger is stiff, the fourth is lacking, as is also the end of the fifth. The hand shows the presence of numerous grafts which have taken, and a few keratoses. The patient was at work at a medical school, and did not submit to an operation until July 5, 1907, at which time it was evident that the forefinger must be amputated, for undoubted epitheliomatous ulcerations were forming in several parts of the dorsum of the finger, notably about the opening into the joint before mentioned. Under ether, the forefinger was amputated at the middle of the first phalanx, a palmar flap of sound skin being reflected backward. Several suspicious areas were excised in other parts of both hands, and skin grafted. Microscopic examination of the amputated forefinger showed five different areas of carcinoma, which owing to delayed operation had infiltrated deeply the subcutaneous tissues, surrounded the digital nerves on the radial side, and caused the extreme pain (see Plate XXXVII., Fig. 16). The columns of epithelial cells had penetrated to the periosteum. As a result of this examination it was decided on July 15 to amputate higher up. The head of the first metacarpal bone was removed and a large flap reflected onto the dorsum of the hand, thus forming a sound stump for approximation of the thumb.

In September, 1908, after eating lobsters, there occurred a general urticaria and both hands became much swollen and the serous discharge was extreme. The patient was confined to bed for ten days, then very gradually the exudate dried up and there followed desquamation. This, however, did not affect the keratotic areas. There have been one or two injuries to the base of the forefinger of the left hand, and to the base of the middle finger of the right hand, which set up extremely painful and obstinate excoriations. For several months there has been an inflamed warty growth over the stump of the little finger of the left hand.

Examination and operation Dec. 7, 1908 (see Plate XXXVII., Fig. 18). Over the head of the ulna, on the left, are two irritated thickened excoriated areas, which are being continually injured by the cuff. Over the center of the hand there is a small, tender, deep ulceration about the size of a split pea. Over the base of the forefinger, and along the ulnar side of the thumb are keratoses cracks and fissures. The left hand was the first operated upon. The warty growth on the little finger was excised down to the aponeurosis and submitted to Dr. Whitney, who reported epithelioma. The question arose as to whether the finger should be amputated at the knuckle or whether more conservative measures were justifiable, owing to the usefulness of this finger. I decided to remove a slightly larger margin down to the aponeurosis, which was then thoroughly cauterized with the Paquelin cautery, The other areas described were deeply shaved, and skin grafted just back of the first interphalangeal joint, and another about the center of the middle phalanx were also excised and grafted. After this operation the remainder of the left hand seemed in good condition. There was, of course, atrophy of the hair, some telangiectases, large areas covered by grafts and cicatricial tissue. The condition of the right hand was as follows: the middle finger is partially flexed and there is hyperextension at the first interphalangeal joint. There is, however, flexion at the knuckle. The worst lesion seems to be excessive hypertrophy and keratosis of the skin, over knuckle of little finger, as shown in the photographs. This spot is frequently subjected to slight trauma. There are similar inflamed and thickened areas on the radial side of the first knuckle, and along the ulnar side of the thumb. The nail of the thumb is deformed and split on the ulnar side of the median line, where granulation tissue and hypertrophied matrix keep up a chronic sore. There exists over the ulnar bone, similar to the left hand, an area of thickened epithelium which is irritated by the cuff. At the base of the middle finger there are several keratoses. The ulnar half of the thumb nail was removed, and with curette and knife the matrix was thoroughly eradicated. The area over the base of the middle finger was excised down to the cicatricial tissue, exposing a small part of the tendon in the median line. All of the keratoses were shaved off with a knife and grafted. It was quite remarkable at this operation to see the hemostatic action of the grafts. Once placed in position, bleeding quickly diminished, and in a few moments practically ceased. Both hands were placed upon splints, the forearm held in position by adhesive plaster, and the grafts covered with small strips of protective tissue. Two keratoses were excised, one from the forehead and the other from the inner part of the upper eyelid.

Dec. 20, 1908. The large and important graft over the knuckle of the middle finger has healed soundly, as have five other grafted areas on the right hand; grafts upon two small keratotic areas failed. The grafts placed on the left hand, however, have not been so successful, owing to hemorrhages underneath them. Only two out of five have taken. The base of the little finger, which was cauterized, has been extremely painful, and is granulating very slowly.

A more careful pathological report showed epithelioma at the site of the excision on the little finger of the left hand, and commencing epithelioma over the knuckle of the right middle finger. All of the other areas excised were negative.

Jan. 20, 1909. In spite of various sorts of dressing the ulceration after cauterization of the little finger has been persistently and exquisitely sensitive and painful, causing loss of sleep and necessitating opiates and, locally, Schleich's solution. To-day egg membrane was tried, and gave such marked relief that several skin grafts were applied as a dressing, though from the character of the granulations it seemed unlikely that they would grow. The pain was immediately relieved, and much to our surprise part of the grafts "took" and slowly extended downward over the ulcer. Progress ceased, however, by February 10, and in view of the extreme pain and danger of recurrence it seemed as if the attempt to save the finger had been a mistake. It was decided, therefore, that if the next attempt at grafting proved unsuccessful that amputation should be done. On Feb 19, 1909, under ether, the ulceration was thoroughly disinfected and carefully shaved down to a smooth base. Thin grafts were used and held in position by protective strips. Several other areas were excised and similarly treated, the majority of which grew, but at the end of two days infection occurred in the little finger, and all of the grafts came away. Pain, however, has been distinctly less after the last operation and, in a few days, epidermis began to grow from the former grafts and the edges of the wound, so that by March 1, 1909, spontaneous healing was complete.

Summary: Commencing with July 10, 1897, I have operated upon this patient under ether thirty-two times, the operations varying in duration from one hour and a half to three hours. At present there remains of his left hand two joints of the little finger, the forefinger and thumb; of the right hand, the thumb, the middle finger, barring part of the terminal phalanx, and one and a half phalanges of the little finger. More than half of the skin of the backs of both hands consists of Thiersch grafts. From the initial dermatitis there has been continual, though slight enlargement of the axillary glands, but no evidence of recent increase in size, or any suggestion of metastases. There was undue and dangerous delay in amputating the cancerous forefinger of the right hand. With this exception, epitheliomatous ulcerations have been excised early. For two months it seemed as if the attempt to save the stump of the little finger was a failure, but in spite of the severe pain the patient seemed unwilling to sacrifice it, owing to the damaged forefinger and the fear that with its possible loss in the future the thumb would have nothing to press against in grasping small objects.

The number of different carcinomata in this one case has been over a score. I have no doubt that general metastases would have taken place long ago had it not been for timely excisions and amputations.

CASE XX. — Personal. F. J. B. (physician); 34 years, Baltimore, Md. Began X-ray work in 1900 and suffered at the start from an acute dermatitis. When this subsided, all finger nails, with the exception of those on the thumbs, came off. After six months' rest the dermatitis cleared up, and all of the nails grew again, except that on the right forefinger; this never reformed. In the spring of 1902, after resumption of work with the X-ray, there was no active dermatitis, but keratotic patches formed on the hands with the appearance of the usual telangiectatic areas. Gradually the nails became deformed. Up to this time there was little pain but considerable stiffness in the hands. The keratoses increased up to 1905, when the first ulcer appeared on the dorsum of the left hand and one on the right forefinger at the junction of the second and third phalanges. These ulcerations were excised and numerous keratoses were removed. The ulcer on the right forefinger returned in a few months and remained about the size of a pea. Numerous attempts were made to heal it as it was exceedingly painful, but these were not successful. The keratosis on the base of the right finger returned after excision and has persisted up to the present time, without ulceration. In 1906, ulceration began in a small keratosis which had been present for several years, situated on the external side of the nail of the ring finger of the right hand. This has since gradually increased in size, extending along the outer edge of the nail, and then upwards upon the dorsum of the finger almost to the first interphalangeal joint. The pain in this ulcer for the last nine months has been very intense, so that for many nights no rest could be obtained. In the fall of 1907 the matrix of the right forefinger nail broke down and assumed the character of a sluggish ulcer, exquisitely tender and painful. The whole matrix was destroyed. At present the dorsum of the forefinger, almost to the joint between the first and second phalanges is occupied by a raw, beef red ulceration without induration. This resembles in a striking degree the ulcerations which occurred on the ring fingers of Case XIX. "The pain is most intense and is best described as a sharp shooting neuralgic pain of the worst character. It lasts two or three seconds and occurs from eight to ten times every minute. When the pain was of this character the ulcer always appeared raw and the granulations very red. Next would come the formation of a slough, when the more intense pain would disappear."

Patient entered the Massachusetts General Hospital on Jan. 19, 1909. Both hands presented typical X-ray dermatoses extending up to the wrists. There were numerous keratoses and a few crusted spots The chief lesions have already been described — the raw ulcerations on the distal phalanx of the first and ring fingers of the right hand. The nail of the middle finger is deformed and there is considerable suppuration about the thin and cracked edges of this nail.

Operation Jan. 20, 1909, under ether. As I feared malignant disease of the forefinger and the ring finger, a small portion from the forefinger ulceration was first removed and given to Dr. Whitney for rapid examination. Though nothing malignant was found, I determined upon amputation of the ends of both of these fingers, owing in part to questionable malignancy, but chiefly because I believed that the grafts, even if successful, would ultimately break down. Accordingly a transverse incision was made down to the bone, through the skin, just below the first interphalangeal joint. The skin was carefully dissected downward, and the bone divided at the middle of the second phalanx; a long palmar flap of healthy tissue being turned backward and sutured in position. This gave a rough resemblance to the crescent of the finger nail. Numerous keratoses and minor ulcerations were deeply shaved from various parts of both hands and skin grafted. All wounds were dressed with protective, and carefully applied pressure held the grafts in firm apposition. There occurred at the site of both amputations a slight degree of infection which delayed healing. By making two parallel incisions at the angle of the nail an attempt was made to extirpate the matrix of the middle finger nail; the pulp of the finger was grafted; of twenty other grafts, eighteen took. The patient was discharged on February 14, with sound union in the amputations and all grafted areas looked well.

On April 1, patient stated that he had been entirely free from pain since leaving the hospital, for the first time in years. About the matrix of the middle finger there is slight suppuration, and from the sides there has been some reformation of soft nail. The grafts are looking very well. After consultation it was decided that this patient should be allowed to continue the use of the X-rays provided he never exposed himself unprotected.

Pathological examination by Dr. Whitney. More careful examination of the tissues removed from this case showed that in both forefinger and ring finger there was undoubted superficial carcinoma; the other areas excised were negative.

CASE XXI. — Personal communication from Dr. L. L. McArthur, of Chicago, Ill. S. C. G. (physician), who combined electrical therapeutics from a static machine with X-ray pictures; he developed his own plates. Chronic dermatitis; ulceration of the terminal phalanges of first and middle finger of left hand; amputation of terminal phalanges. Later, there developed a typical epithelioma on the remains of the left index finger, and numerous keratoses. The finger was amputated and found to be carcinomatous. Keratoses excised and grafted; axilla thoroughly dissected. No glands found to be involved. Patient well one year after.

Summary: Epithelioma of left index finger; amputation; dissection of axilla. No glands involved. At present no evidence of recurrence Prognosis good.

CASE XXII. — Personal communication from Dr. L. L. McArthur, of Chicago, Ill. W., of Chicago; probably the first X-ray burn on record. Eleven hours' exposure within a period of three days. Huge burn extending from the symphysis to the chin, the healing of which required several plastic operations. In July, 1908, having been well in the interim, he was thrown from his pony and injured the cicatrix. The ulcer refused to heal and carcinoma rapidly developed, until an area 10 x 4 inches had become involved. At operation, with a wide margin, the cancerous skin was removed, as well as the anterior sheath of the rectus, part of the rectus muscle, and in parts even its posterior sheath. By undermining the skin laterally, beyond the axillary line, the wound was closed anteriorly, and though infection occurred, healing is now nearly completed, Dec. 11, 1908.

Summary: Severe X-ray burn in 1896, involving the chest and abdomen; plastic operations. Latent period "eleven years;" extensive carcinoma of abdominal wall; radical excision.

CASE XXIII. — Personal communication from Dr. Charles H. Bowen, of Columbus, O. L. M. E (physician); has been doing X-ray work for the last ten years. Nine years ago he developed a dermatitis which gradually increased in severity until ulcers developed. These ulcers were curetted from time to time, and skin grafts were tried, but seemed of no permanent benefit. The grafts would take, but in a few months would break down, and the ulcerations would be as painful as ever. His phalanges were amputated on a number of occasions, until he finally lost three fingers from his left hand and two from the right. Several months ago he developed an ugly ulcer on the back of his left hand, which gradually became an epithelioma. This was curetted on several occasions, but without effect. The remains of the right hand is so painful that it has to be kept continually covered in a layer of cotton in order to avoid the slightest injury. In April, 1908, the left forearm was amputated at the middle. In December, 1908, no evidence of recurrence on the left. The right hand, which had quite a number of very suspicious looking ulcers, has been gradually getting better, under large amounts of iodine, though not necessarily caused by it. X-ray work and medicine have been given up. There is no evidence of metastases.

Summary: X-ray worker; persistent ulcerations and deep epithelioma necessitating amputation of left forearm at the middle. Persistent ulcerations of right hand required amputation of two fingers. Recently, ulcerations on the back of the hand have improved, while taking iodine and giving up work.

CASE XXIV. — M. W.; X-ray manufacturer; static machines; has suffered for years with the severer grades of dermatitis; recently multiple epitheliomata have developed on the hands and fingers. After several operations there remains on the right hand the thumb and forefinger; on the left hand the thumb, index, and middle fingers — the fourth and little fingers, with their metacarpal bones, have been removed. There are other lesions on the face and back.

CASE XXV. — F. D. A. (physician); radiologist, Rochester, N.Y. Amputation of left hand; X-ray carcinoma; Journal American Medical Association, Dec. 12, 1908. Personal communication from Dr. J. Nevins Hyde and Dr. Ormsby of Chicago; two private patients never reported.

CASE XXVI. — O. R.; severe X-ray lesions after treatment for plantar keratodermia; epithelioma; excisions of soles of both feet; Krause grafting successful.

CASE XXVII. — P. J. S.; epithelioma of back of hand, following X-ray lesions.

CASES XXVIII., XXIX., XXX., XXXI. — Four cases of carcinoma, operated upon by Dr. Sick of Hamburg, abstracted from an article by Dr. E. Schümann in Archiv. für Klinische Chirurgie, 1907, Band 84, page 860.

CASE XXVIII. — X-ray operator; cracks and fissures of fingers and on dorsum of hands, which were excessively painful and did not yield to any conservative treatment. These warty growths of epithelium were finally excised and sutured. Unna examined the specimens and pronounced them as beginning epitheliomata. The tissue was so friable that the sutures did not hold, and it was difficult to make satisfactory sections. The wounds healed by second intention and remained well thereafter.

CASE XXIX. — Patient, X-ray tube maker. Usual pigmentations of the skin of face and arms; typical changes of finger nails. In addition, two prominent keratotic growths on the back of one hand. These were excised and pronounced by Dr. Fraenkel to be epidermoid carcinoma.

CASE XXX. — Young physician; severe lesions on fingers and backs of hands. On the right forefinger a chronic paronychia, and on the back of the hand a small ulceration. Usual treatment without avail. The pain was extreme, often keeping the patient awake all night. Evulsion of the nail was not beneficial. The terminal phalanx was amputated and the ulcer excised and skin grafted. The graft died, but satisfactory healing took place eventually. Pathological examination showed typical skin cancer.

CASE XXXI. — Man, 32 years, who after three years of X-ray work suffered from severe lesions on both arms, breast, neck, and face. In 1901 there began a slowly growing ulceration of the back of the right hand, which by the middle of 1902 had become a gangrenous epithelioma; glands enlarged at the elbow and in the axilla. Amputation at the shoulder; axillary glands removed, and found full of squamous celled carcinoma. Sound healing. In December, 1904, a typical cancer of the lower lip and another of the angle of the mouth were excised, as was a suspicious lesion on the back of the left hand. In March, 1905, a growth of the cheek was removed, which was pronounced by Unna to be a sarcoma. In September, 1905, excision of the right lower jaw for carcinoma. Recurrence involving the tongue and the adjacent cheek was present in February, 1906. Death soon followed.

CASE XXXII. — Patient operated upon by Professor Trendelenburg. The specimen was carefully studied by Dr. Schümann. Man, 39 years; unusually exposed since 1896 to the X-rays — he used his own hands to test the tubes — four years later, developed a dermatitis of both hands. The skin became rigid, dry, and swollen; the nails brittle with chronic onychia. This condition lasted two years. In 1902 he became worse. On the right hand there developed yellowish, horny growths, particularly over the knuckle of the third finger. In 1906 the removal of this keratosis left behind an ulcer, which in two months grew to the size of a mark piece with indurated edges. The epitrochlear glands were enlarged; the axillary glands not palpable. In February, 1907, the ulcer was radically excised and skin grafted; healing uncomplicated. No mention is made of the treatment of the enlarged epitrochlear glands. Examination of the ulcer showed typical epidermoid cancer.

CASES XXXIII., XXXIV., XXXV., XXXVI., XXXVII. — The following cases occurring in Great Britain are abstracted from a paper by Mr. Foulterton in the Transactions of the Pathological Society, July, 1906, and from personal communication from Dr. Cecil W. Rowntree, and a paper by him, published in the Archives of the Middlesex Hospital, Volume XIII., July, 1908.

CASE XXXIII. — X-ray work in 1897, at the age of 38. In two years, loss of tactile sensation in fingers. In May, 1903, severe dermatitis. Resulting ulcers took four months to heal. Warty bodies next developed on the dorsal aspect of index and second fingers. A similar acute attack in December, 1903. An ulcer on the index finger refused to heal and increased in area and depth; in September, 1904, the finger was amputated. In 1906, a small superficial ulcer on the middle phalanx of the middle finger persisted, and was excised and grafted. Examination of the ulceration, for which amputation was done, showed a typical epithelioma with cell-nest formation, which invaded the underlying bone. Examination of the ulcer excised in 1906 shows absence of elastic tissue, thickening of the epithelium, early keratinization and plasma cell infiltration; a precancerous condition.

CASE XXXIV. — Man, 60 years; engaged for some years in the manufacture of X-ray tubes. For painful fissure over the last interphalangeal joint of the first finger, excision was performed. Microscopic examination showed early but undoubted squamous cell carcinoma.

CASE XXXV. — Man, 40 years. The third finger showed a glossy appearance of the skin, which was thin and adherent to the underlying structures. The nail had entirely disappeared, except for a small portion at one edge. There were no cracks nor warts, nor any actual ulceration anywhere, but at the base of the finger was an area where the surface epithelium showed a slight irregularity. This finger was amputated at the metacarpo phalangeal joint, and the metacarpal bone subsequently dissected out. Examination of the indurated skin showed the presence of a malignant growth, infiltrating the deeper structures; a typical squamous cell carcinoma, with well-marked cell-nest formation. The axilla was cleaned out, but the enlarged glands showed no evidence of metastases.

CASE XXXVI. — Man, 42 years; engaged for several years in the manufacture of X-ray apparatus. For two years the left hand had been affected, and eighteen months previously a growth had appeared on the middle finger; surgical treatment had been refused. Finally the left middle finger became very greatly enlarged by an irregularly lobed new growth, which was ulcerating on the surface and very foul. There were a number of irregular, pigmented warts; no enlarged glands. The finger was amputated through the proximal end of the shaft of the third metacarpal bone; good recovery. Since this operation in 1905 there has been no evidence of recurrence in the hand, but there are persistent ulcerations upon the chin and over the upper part of the sternum. Microscopic examination of the growth shows that it is composed of small and slender columns of eyelets of squamous cells, irregularly distributed and separated from one another by a well-marked connective tissue stroma, composed of large cells with large nuclei. Mitotic figures are present. "Of special interest in this ulceration is the striking appearance of the connective tissue stroma, the individual cells being of a size and character not usually met with. Their large size and great number produce the appearance as if the small eyelets of epithelial cells were dispersed throughout granulation tissue, and suggesting the view that the connective tissue cells themselves are endowed in these instances with a special activity." None of the cells were found to be multinucleated. An X-ray of the finger removed showed a total disappearance of the bony tissue of the first and second phalanges and suggested further study by the X-rays of such hands. In other instances, also, marked atrophy of the bony tissue was noted, and in one case absorption had taken place, giving the appearance of a punched-out area in the middle phalanx.

CASE XXXVII. — Man, 38 years; seen in July, 1905. Had been working with the X-rays for six years. On account of trouble with his hand gave up work in 1902. The skin of both hands was thin, glossy, and pigmented; usual telangiectases. On the proximal phalanx of the right middle finger was a growing ulcer about one inch in diameter. There were two other smaller ulcers, which showed no evidence of healing. No enlargement of glands. The finger was amputated with nearly the whole metacarpal bone, and the ulcer over it. The other ulcer was excised and grafted. Patient remained well for a year, when a small ulcer appeared in the scar; this healed, however, eventually. A few months later a scab formed over the scar, which was gradually raised by a prominent growth about the size of half a cherry, which rose dome-like above the skin. This growth was bright red in color; of firm consistency. It was removed, and the resulting wound covered with a Thiersch graft. At present, May, 1908, there is no recurrence. Microscopic section of the original ulcer revealed a keratinization and a squamous cell carcinoma, in which the cell nests are numerous and well developed. Examination of the second growth showed that it consisted entirely of young granulation tissue, which replaced the epidermis and corium for a limited area, the epidermis gradually merging into and disappearing in the granulation tissue. "The cells composing the granulation tissue are unusually large and well developed, and possess large, round, oval nuclei, containing much chromatin and staining deeply; in fact the appearance of these cells are strikingly similar to that of the cells which formed the stroma of the new growth in the previous case. The elastic tissue is disappearing in a narrow zone below the epithelium with moderate plasma cell infiltration.

Summary: Five cases of carcinoma, developing with a long latent period, in cases of chronic X-ray dermatitis. In all but one, early operation. One case neglected, but no involvement found in the axillary glands. In two instances, attention is called to an unusual form of granulation tissue in which the cells are unusually large and contain large, round, or oval nuclei, and stain deeply.

CASE XXXVIII. — H. E. (physician); amputation of arm for epithelioma of hand.

CASE XXXIX. — At Leeds, England; amputation of arm for carcinoma.

Fatal cases, X-ray carcinoma.

CASE XL. — B. (physician), England; X-ray carcinoma of hands; general metastases and death.

CASE XLI. — W. C. E. (physician). Personal communication from Dr. Nevins Hyde of Chicago. X-ray cancer of hand; general metastases and death.

CASE XLII. — W. (physician), Rochester, N.Y. Personal communication from Dr. W. B. Coley of New York. For five years, had been using X-rays continuously. Ulcerations present for six months on the backs of both hands, specimens from which were examined by Professor Welch of Johns Hopkins Hospital and found to be undoubted epithelioma. The carcinoma had deeply involved the metacarpal bones of the index and middle fingers. Amputation of right hand above wrist, and thorough excision of ulceration on the left hand, Oct. 10, 1904. Subsequent recurrence in axilla and liver; death.

CASE XLIII. — C. D. Personal communication from Dr. Samuel Lloyd of New York and Dr. W. B. Graves of East Orange, N. J. This patient was very seriously burned on both hands years before in working with X-ray tubes. He was treated at many different hospitals. Skin grafting was tried without result, until finally epithelioma developed on the right hand and on the base of the little finger of the left hand. At the time of Dr. Lloyd's first examination the right hand was infiltrated with carcinoma and the axillary glands were involved. The arm was amputated at the shoulder joint, and the glands above and below the clavicle removed. Amputation of the left hand was advised but refused. This was Aug. 8, 1902. The ulceration gradually increased in size and depth and the left arm was amputated by Dr. Graves on March 16, 1904. Death followed from mediastinal recurrence in October, 1904.

CASE XLIV. — B. F. (female), of San Francisco, Cal. Personal communication from Dr. Childs Macdonald. Patient was a pioneer in X-ray work. In 1903 her hands commenced to show signs of X-ray dermatitis, the nature of which was not understood, and attributed to chemicals used in developing the plates. Patient worked twelve hours a day, without protection. In the early part of 1904 she came for the first time under treatment. The fingers of both hands were badly ulcerated; chiefly the tissues over the middle phalanges and the middle joints; all the nails were affected, the surfaces presenting ulcerated, warty condition, the warts assuming the form of necrogenica, healing alternating with ulceration. All the secreting glands and hair follicles were destroyed, so that the skin was hard and dry, and cracked easily. All sorts of treatment of ointments and washes were without permanent benefit. In November, 1904, a wart appeared on the index finger, near the terminal phalanx, which was very raw, and grew with great rapidity. A portion was removed, under cocaine, and submitted to Dr. Rifkogel, who reported a branching papilloma, with a tendency to downward growth, and advised amputation. This the patient refused, so the growth was freely excised and healed healthily. A few weeks afterwards, however, a nodule appeared near the scar, which pushed up through the epithelium, broke through it and appeared exactly the same as growth No. 1. The glands in the axilla were found somewhat enlarged. Radical operation was again refused, until after Christmas, when axilla was freely opened and the whole space cleared of glands. Pathological examination of these showed undoubted involvement with epidermoid carcinoma. In the course of a week, more nodules appeared just below the acromioclavicular articulation. It was decided that the only chance of recovery lay in amputation at the shoulder. Accordingly on Jan. 27, 1905, after preliminary ligation of the subclavian in the third part, the arm and scapula with the clavicle were removed. The patient rallied well from the operation, and the wound healed rapidly, but about the end of April, 1905, a recurrence took place at a point representing the position of the inferior angle of the removed scapula. This was excised and found to be carcinoma. Slowly, and then rapidly, other nodules appeared, until ultimately the whole of the vertical scar, corresponding to the vertebral border of the scapula, became involved. Metastases took place in the pleura and lungs, and the patient died on Aug. 3, 1905.

On reading this history it is obvious that the patient by refusing early radical surgical treatment jeopardized her life, though it is of course possible that metastases had occurred early.

Case XLV. — F. (physician). Personal communication from Dr. L. L. McArthur of Chicago, dated April 26, 1907. Patient was the first photographer to develop X-ray plates in America. "He early developed the chapped and fissured hands which go with the exposure to the ray, especially in those who combined the development of their plates with their X-ray work. For the last three years he has been having, from time to time, minor surgical procedures in the way of removal of a phalanx now and then, until three fingers of the right hand and two on the left had been sacrificed. After various plans of local treatment and a trip to Europe in search of remedial agents, he came to me for the first time with an enormous axillary involvement. This was indubitably squamous celled carcinoma. The radical operation with removal of portions of the pectorales and a dissection of the axilla en bloc failed to effect an arrest of the trouble. Recurrence took place very promptly, and within three months again filled the axilla, and involved the supraclavicular glands. Deeming it hopeless to subject him to further surgical interference, trypsin injections were given, with a resulting liquefaction of the axillary mass to a nonpurulent, sero-sanguinolent fluid, containing immense masses of epithelial cellular detritus, as was shown on opening and draining the same. After lingering for six weeks he finally succumbed to a multiple metastasis on April 23, 1907.

Case XLVI. — R. V. W. (physician); X-ray machine manufacturer; has used both static and coil machines continuously from their beginning, subjecting himself to countless fluoroscope examinations. His skin, both front and back, for a number of years, has been covered with characteristic lesions, many of which have recently become epitheliomata. He was operated upon for a carcinoma of the cheek, by the actual cautery, down to the mucous membrane, and a thorough removal of epitheliomatous glands on both sides of the neck. He had also epitheliomata of both hands and some on the back. Patient died in April, 1908, of extensive metastatic carcinoma of the liver.

CASE XLVII.—J. B. Personal communication from Dr. Russell S. Fowler and his house surgeon, Dr. C. H. Criley, of the Brooklyn Hospital, New York. Man, 33 years; a glass worker by occupation, since the age of fourteen. Soon after the discovery of the X-ray, patient became interested in the manufacture of the tubes. His work was chiefly in the testing-room, where he worked over a bench, waist high exposed for one to four hours a day. In 1900 he noticed a few small, warty growths on the fingers, knuckles, and backs of hands. In 1904 patient gave up business on account of general poor health, at which time he noticed a peculiar erythema with a slight tendency toward hard, warty nodules over the face, neck, and anterior trunk, above the waist line. The keratoses were still very small, the largest not more than a quarter of an inch in diameter. He has some on the face removed with the actual cautery and silver nitrate. The growths rapidly recurred and increased in size, becoming soft and slightly ulcerated.

Examination, April 19, 1907. Marked erythema and mottling of the skin of face, trunk, and arms. Many scattered, hard, flat papules. A small cutaneous horn on the forehead and several smaller ones on the chest. Large epithelioma over the metatarso phalangeal joints of index and middle fingers of left hand. Deep ulceration and small ulcerations over some of the joints of the right hand.

Operation: amputation of first and second fingers with heads of metacarpal bones of left hand. Removal of numerous epitheliomata. Patient refused more radical treatment and would only allow curetting of the carcinomata on the right hand.

Nov. 11, 1907. The growths on the right hand had increased in size and fused, but were not deep. Rapid recurrence at site of amputation of fingers of left hand; only curettage was permitted, followed by an unsuccessful attempt at skin grafting.

March 11, 1908. Since last examination, rapid progress of the disease, involving the base of the thumb and the metatarsal bones of the third and fourth fingers; no evidences of glandular enlargement. Amputation at junction of middle and lower thirds of left forearm; amputation healed by primary union. Another curettage of the right hand.

April 20, 1908. Another attempt was made to benefit right hand by curettage and skin grafting; unsuccessful. Discharged on June 14, with cancer slowly spreading on right hand. No evidence of granular enlargement anywhere. By Aug. 8, 1908, however, the general condition had become poor, with marked loss of weight; epitrochlear and axillary lymph nodes on the left enlarged; no recurrence in stump. Epithelioma of right hand had begun to spread rapidly, and had involved deeply the metacarpo phalangeal areas of the first and second fingers. These fingers were accordingly amputated on August 11, and the wound skin grafted. The left epitrochlear and axillary glands increased very rapidly in size, forming great masses which were extremely painful. On Sept. 25, 1908, interscapulo thoracic amputation was performed, and a small epitheliomatous ulcer removed in the region of the umbilicus. The amputation wound healed very slowly, showing evidence of recurrence. Patient died Nov. 7, 1908, of general carcinosis.

Pathological report. Glands above clavicle carcinomatous. The lymph nodes in the mediastinum and about the bronchi were enlarged to about the size of a fist. There were nodules in both the parietal and visceral layers of each pleura. Some nodules in both lungs. Nodules also in kidneys and adrenals. Numerous carcinomatous areas in the liver and one in the wall of the stomach.

Summary: In 1897 began work with the X-rays, testing the tubes for several hours a day. First noticed erythema and warty growths in 1900. In 1905, keratoses and warts had formed on both hands, chest, and face. First carcinoma developed and required amputation of two fingers of the left hand in April, 1907; similar growth curetted on right hand. By March, 1908, rapid extension of the disease necessitating amputation of left forearm; curettage of epithelioma on right hand. August, 1908, involvement of epitrochlear and axillary glands. Aug. 11, 1908, amputation of fingers of right hand. Sept. 25, 1908, amputation at shoulder. Death on Nov. 7, 1908; general carcinosis.

On reading this history it is obvious that death was caused by the patient's refusal to subject himself to early and radical operation. The amputation at the shoulder was not performed until after general metastases had taken place.

While on reading the histories of these cases one cannot fail to be struck by the inevitable and progressive evolution of the disease from the milder grades of inflammation to ultimate carcinomatous degeneration, there appear, fortunately, a few exceptions to the general rule, which careful inquiry from my own and other cases have developed; where through the years, in spite of persistent lesions, there has been a gradual improvement in the general condition of the hands, as if the natural reparative power of the skin had begun to assert itself. This is analogous to a few recent cases of improvement in the genital glands, after protracted loss or abeyance of function. The degree to which this return toward normal may develop must depend upon the presence or absence of continued irritations, and the depth or degree of the original damage to the skin and subcutaneous tissues.

Pain. — The amount of pain which these patients suffer is variable, though usually extreme. From my experience and personal communications from patients I believe that the agony of inflamed X-ray lesions is almost unequalled by any other disease. Its character has been described, and varies with individuals. While morphia has been used in some of the cases, it is really surprising to find how many have borne their pain without resorting to its habitual use. The pain seems to be due, in some instances, to the exposure of irritated nerve endings in a raw ulcer, like the "erethystic" varicose ulcer of the leg, to suppurating nail beds, irritated by small pieces of nail, and, in acute burns, very probably to a definite neuritis. In the more chronic cases I believe that the microscopic sections show quite clearly that the pain is caused by the cicatricial contraction in the thickened corium, compressing the nerves. In one case of mine (XIX.) the nerves were shown to be actually involved in an infiltrating cancer. At another time, the lateral digital nerve of the little finger was exposed at the base of a slowly-healing sore; sensitiveness and pain were intolerable, but ceased as soon as the bare nerve was covered with granulation tissue and skin. Without regard to the particular cause of the pain, in either the acute or chronic cases, adequate excision of the lesions has invariably given immediate relief. In Cases IV. and XV., though differing so far as one could see in no respect from other very painful ulcerations, pain was remarkable for its absence throughout the whole course of the disease. Rupture of the dilated capillaries is of frequent occurrence and always accompanied by extreme, radiating pain, until the extravasation works to the surface, forms a black spot, and is thrown off in the process of desquamation, when the pain ceases. Not infrequently, these minute hemorrhages form the sites of subsequent ulcerations.

Acute infections arising in the fissures or inflamed nail beds, keratoses or ulcerations, or coincident injuries are responsible for much of the disability associated with such hands, always rendering more painful lesions which were painful enough before.

The palliative treatment of these chronic X-ray lesions must be left in a great measure to the personal experience of the individual. Relief from pain and improvement often take place under remedies which in another patient are most painful and harmful. In general, dry treatment, when it can be borne, combined with protection has seemed to be most beneficial. Maceration should be avoided. Pure benzoinated lard I have found the most valuable ointment; ichthyol combined with lanolin occasionally useful. At times, local anesthetics must be used; preferably a weak solution of cocaine upon two layers of compress cloth. Orthoform I am convinced should never be used, owing to its tendency to produce ulcerations. Of the washes, simple salt solution or aluminum acetate are valuable. If infection is present, citrate of silver (1–6,000) has been very useful in most of my cases. Alumnol (1–200) has proved a most satisfactory antiseptic and astringent. The keratoses may be softened with a solution of caustic soda and shaved down with a sharp knife or pumice stone. If they are scratched off, bleeding is profuse, and ulceration not rare. The actual cautery which several have used, while temporarily destroying the condition, seems to invite recurrence on a larger scale. Sparking with high tension current has been tried by various radiographers with temporary improvement, but no permanent benefit. With the non-surgical methods of destroying keratoses, I have had no personal experience. Dr. Whitehouse of New York prefers liquid air, while Dr. Pusey of Chicago is well satisfied with carbon dioxide snow. Both of these milder methods have the advantage over excision of causing no detention from business, and possibly of acting only upon the abnormal cells, without affecting normal tissues.

After months or years of treatment, or neglect of treatment, carried out with more or less relief upon various lines, the early X-ray worker usually finds that while the general condition of the hands may have improved somewhat, either certain keratoses recur after removal, or the nail beds are affected, or that after healing and breaking down several times a particular ulceration finally refuses to heal. The lesions are chronic, moderately severe, recurring, eventually persistent. The first question usually asked is, "Should X-ray work be given up?" While it is obvious that to do this would be conservative, I feel personally convinced that no harm results from X-ray work carried out with all modern precautions. It is easy to give this advice, but only in exceptional instances is the advice conscientiously carried out. The second question, "What is the danger of cancer?" Provided ulcerations have not persisted for more than three months, or have not occurred at the site of an inflamed keratosis, I believe it to be slight.

The surgical treatment of X-ray lesions. — Acute and sub-acute burns: By this I mean the results of one or at the most a few protracted exposures such as occurred in Case II. The severe symptoms develop from a few hours to a few weeks after the injury, and are accompanied by extreme pain. The area apparently involved gradually shrinks, leaving behind, eventually, evidence of the actual damage done. Depending upon the depth of the lesion there is desquamation, blistering, ulceration, or deeper death of the tissues. In all degrees we have the abundant formation of a very tough and adherent fibrin with great pain upon its removal; this condition is well shown in Case VIII., and is peculiar to X-ray lesions. For a time, at least, the deteriorated tissues seem to be in unstable equilibrium, unable to slough or to repair themselves. Pain is extreme and for its relief the tendency is to perform an early excision, but it is most difficult to determine how much tissue should be excised. While in Case II. immediate relief of the pain followed operation, and the excision at the time seemed adequate, the wound failed to heal and further necrosis occurred in the margins and base of the wound. In general, then, except for the pain, I would advise conservative treatment of these acute or sub-acute lesions until what would correspond to a " line of demarcation " has been established. Excision with skin grafting should be preferred to plastic operations. If the graft fails to grow owing to continued necrosis, later grafting may be done after the wound has granulated.

Fissures and thin skin over the knuckles. — The treatment, whether conservative or operative, must depend upon the individual experience of the operator and the choice of the patient. If skin grafting is successful, there can be no doubt that the result will be most satisfactory, and to my mind should be advised.

Keratoses. — Until recently, my personal experience led me to believe that simple, uninflamed, non-ulcerating keratoses were benign, but I am now convinced that there are exceptions, and that all keratoses which show a tendency to dip below the level of the surrounding skin should be regarded as suspicious. Microscopical examination of many of those which I have removed showed all stages from a perfectly harmless condition to beginning invasion of the corium. I have had no experience with liquid air or carbon dioxide snow, but have found their removal by operation very satisfactory. If situated upon the dorsum of the hand where the skin is lax, excision and suture is satisfactory. If upon the fingers, especially over the knuckles, excision and skin grafting should be practised. If superficial, excisions well into the corium will be adequate when a thin Thiersch graft can be applied. If primary grafting fails, I am convinced from several observations that thorough removal of the diseased tissue promotes spontaneous healing from the edges. It has been suggested that the irritation of the graft excites the surrounding skin to the formation of thickened epithelium, but it has seemed to me that if recurrence of the keratoses takes place at the margins of the graft that this is more reasonably explained by inadequate excision. If the keratosis suggests malignancy, the whole thickness of the skin should certainly be removed.

Pachydermia with telangiectasis. — The proper treatment of this condition is extremely difficult to determine. In Case XV., where the whole front of the chest was affected, and in VIII. where the anterior abdominal wall was in a similar condition, with undoubted epithelioma in certain unsuspicious localities, treatment must depend upon the individual indications. In a case like XV. the patient must be watched with care; in one like Case VIII. it soon became obvious that all of the damaged tissues would gradually slough unless removed by operation. The histological examination of such tissue shows that the main arteries are pervious, but that there is both marked endarteritis and endophlebitis, which unless collateral circulation is formed will probably lead to subsequent anemic necrosis.

Paronychia and onychia. — Probably the most painful of the X-ray lesions, and one of the most persistent. If disinfection and dry treatment does not bring about healing in a reasonable length of time, extirpation of the nail is clearly indicated. If healing then does not take place, the whole nail bed, both at the base and on the sides should be destroyed. Owing to the difficulty of distinguishing between nail-forming membrane and the insertion of the extensor tendon into the terminal phalanges, subsequent re-formation of portions of the nail is not uncommon. Amputation with certain cure is often too long delayed in this condition.

Ulcers. — I have learned to dread a slowly developing ulcer, in chronic X-ray lesions, which may or may not be painful; the edges may be slightly raised and a little indurated, or soft and flat. Nothing but the persistence of the lesion really suggests malignancy. In Case XVII., though an intermittent ulceration, never completely healed, had persisted for seven years, only beginning carcinoma was shown by the microscope, while a very malignant tumor, either cancer or sarcoma on the forehead progressed very rapidly within half a year. I have been informed that ulcerations have been open for years in the same spot in two cases, and then entirely healed. Such variations in the tendency to malignancy must be taken into consideration in advising radical treatment. On the other hand, pain and disability, not the fear of malignancy, is often an indication for operation. Even rapid histological examination may be unreliable, for twice I have found undoubted cancer, after amputation on the clinical evidence, when a small piece microscopically examined was negative.

Treatment of ulcers, benign and malignant. — If an ulceration becomes exquisitely sensitive and painful, raw and beefy red, whether or not the base is indurated, such as occurred in 1902 in Case XIX., and recently in Case XX., early amputation should be done. As a rule amputation at the knuckle of a finger, upon which a carcinomatous ulcer has developed, will be found to be sound and conservative treatment; but when one finds that multiple lesions upon both hands have been treated, and more will probably occur in the future, in view of the possible reparative power of the skin, and the relatively benign character of these epitheliomata, if treated early, it seems, so far as my experience goes, that conservative treatment (by which I mean local excisions or minor amputation) will probably be adequate to control metastases. Although since 1902 Case XIX. has developed very many carcinomata, and twice at least operation has been too long delayed, there is as yet no evidence of increasing glandular enlargement, which surely would have occurred long ago, except for the timely operations. It is not unlikely that this relative immunity may be partially explained by the obliteration of the adjacent lymphatics, which has been induced by the X-rays. It must become a question, finally, as to how close to the wind the patient and surgeon are willing to sail. For example: this same Case XIX. developed an epithelioma on the stump of the little finger. This he did not wish to sacrifice, as the forefinger was maimed, so after thorough excision, down to the aponeurosis, the actual cautery was thoroughly applied. Healing was most painful and slow, yet at present, six months after operation, there is no evidence of recurrence and the finger is soundly healed. One case has remained well for two years and a half, after thorough dissection of the axilla for cancerous involvement; one of my patients is well a year and a half after a similar operation. In those cases, where there have been many epitheliomata on the hands and fingers, it must be a serious problem as to whether amputation at the wrist, which would practically guarantee a cure, would not be sound surgery; yet I know of no X-ray operator in my experience who would make this deliberate choice.

Skin grafting. — I have been so successful in the treatment of these cases by Thiersch grafting that I personally advise it in all of the more severe X-ray lesions. I have not used Wolff grafts, although some of the Thiersch transplantations have been so thick as to practically amount to the same thing. After two failures in using skin for these grafts from parts to some degree affected, I am convinced that sound skin never subjected to the X-rays has more vitality. Heterogeneous grafts, in the few instances in which I have employed them, have been failures. The disinfection of these cases before operation is a difficult problem, for as open ulcerations invite sepsis in the grafts we would prefer thorough germicidal treatment; but this, if thoroughly carried out, usually results in so much irritation that the subsequent discharge of serum is excessive. I have preferred, therefore, mechanical rubbing with a sponge and salt solution, followed by carbolic acid solution (1–50) for a few moments. If the skin is unbroken, ordinary methods are applicable. A tourniquet should always be used, for bleeding is free and dissections must be delicate when made over the joints, tendons, or aponeuroses. After the removal of the diseased tissues, the tourniquet should be removed, and the hands elevated until bleeding has stopped spontaneously. I have seen little benefit from peroxide, and have never dared to use adrenalin. In some cases the hemostatic action of grafts quickly placed upon an oozing surface has been remarkable. If possible, excisions should have bevelled edges, and the graft should be cut thick in the middle, thinning out towards the periphery, so that it will accurately fit the wound. Firm pressure must be applied for the first twenty-four hours to prevent oozing and hemorrhage. In order to insure even pressure after operation, and to avoid danger of detachment of the grafts by slight motions of the etherized patient, the hand and fingers must be firmly fixed to a well-padded splint by adhesive plaster before any of the grafts are applied. This is often a painstaking procedure, owing to the various deformities and irregularities in the lengths of the fingers between which gauze must be placed to make them comfortable. The grafts are dressed with small pieces of protective placed in an imbricated fashion, and stuck by blood to the palmar surfaces of the fingers; the protective should be perforated. After sufficient gauze has been placed over the protective to make an elastic dressing, firm pressure should be made directly downwards by series of opened gauze sponges which are tied on the palmar surface of the splint. By this manœuvre, detachment of the grafts is prevented, as I have frequently seen when a roller bandage was used, causing gradual lateral creeping of the grafts. After twenty-four hours the protective should be removed with great care, and if the pain is not too severe the grafts should be exposed to the air in a cage. If such exposure cannot be borne, benzoinated lard is the least macerating ointment which I have found. Blisters are punctured as fast as they are formed. If these punctures scab, and serum accumulates under the graft, dry treatment is contraindicated. At the end of three days the hands are bathed in warm salt solution or citrate of silver; while under water small tabs of skin, etc., may be very readily removed, as in making a wet dissection. In ten days the grafts are soundly healed, when dry treatment with occasional lanolin ointment is all that is necessary. The patient must be kept in bed for four or five days with the hands elevated. If the grafts owing to infection or from lack of blood supply do not take over the aponeuroses, a subsequent grafting may be done when the wound granulates, provided spontaneous healing does not take place.

SUMMARY.

With the more general recognition of the existence of these X-ray carcinomata, it seems clear that such early conservative treatment as I have outlined will be adequate to prevent metastases, for a careful study of the fatal cases shows that in eight of them inadequate treatment, either in point of time or extent of operation, due almost always to refusal of the patient to submit to the treatment suggested, was the cause of the fatal issue. In the case of Sick, and also of Wagner, coincident development of epithelioma on the face or other parts of the body made adequate surgical treatment more difficult.

[In my former paper I was indebted to Dr. J. H. Wright, Director of the Clinical Laboratory, and to Dr. W. F. Whitney, Pathologist at the Massachusetts General Hospital, for the microscopical reports of my cases. Dr. C. J. White, Dermatologist to the Hospital, made an exhaustive study and report of the numerous sections removed from Case XIX., while both Dr. Wright and Dr. Whitney have examined the material from many of my subsequent cases, and Professor Mallory of the Harvard Medical School has given me his valuable opinion on two cases. Most of the pathological specimens have been sent to Dr. S. Burt Wolbach of the Bender Hygienic Laboratory in Albany, who is making a special study of X-ray lesions and their causative relation to carcinoma. As his contribution will be published in conjunction with this paper, I devote myself chiefly to the clinical side of the subject, leaving to him a more careful description of the pathological lesions found in the less advanced cases, and an estimate of their value in a theoretical attempt to explain the etiology of cancer, or at least the conditions which immediately precede its development.]

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2. Karl Lindenborn. Same journal, 1908, lxxxix, Heft 2, 385.
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DESCRIPTION OF PLATES.

PLATE XXXV.

FIG. 1. — Case V. Precancerous ulceration. Dr. W. H. M. Condition before and ten days after operation.

FIG. 2. — Case VIII. Miss H. Huge X-ray ulceration with epitheliomatous nodules, as marked in ink square; after first operation.

FIG. 3. — Miss H. Condition after second operation with outline of extent of excision with subsequent healing.

FIG. 4. — Case IX. M. K. K. 1901; note pachydermia.

FIG. 5. — Same, 1903. Note ribbing of nails, rhagades, and superficial ulcerations.

FIG. 6. — Same, January, 1908. Carcinoma base of ring finger, and over terminal phalanx of middle finger of left hand. Keratosis right hand.

PLATE XXXVI.

FIG. 7. — Case XV. F. H. S. Epitheliomatous ulcer back of hand, with numerous keratoses and telangiectases.

FIG. 8. — Same. Low power; carcinoma of hand.

FIG. 9. — Same. Telangiectases of chest with carcinomatous nodule outlined in ink circle.

FIG. 10. — Same. Low power; nodule chest; epidermoid carcinoma.

FIG. 11. — Same. Keratosis nose, high power. Note extraordinary number of mitotic cells.

FIG. 12 — X-ray ulcer leg. Case III.

PLATE XXXVII.

FIG. 13. — J. G. Case XVIII. Recurrent growth of finger, pushing through skin graft.

FIG. 14. — Section same case, low power. Note encapsulated growth and small carcinoma on right.

FIG. 15. — High power of same growth. Pathology described by Professor Mallory, under Case XVIII.

FIG. 16. — W. J. D. Case XIX. Condition of hands, June, 1905. Epithelioma base middle finger, right hand.

FIG. 17. — Same case. Condition of hands July, 1907. Epitheliomatous ulceration forefinger. Flexion owing to destruction of extensor tendon over head first phalanx.

FIG. 18. — Same case. Condition November, 1908. Epithelioma base little finger, left hand. Malignant keratosis base middle finger, right hand.

PLATE XXXVIII.

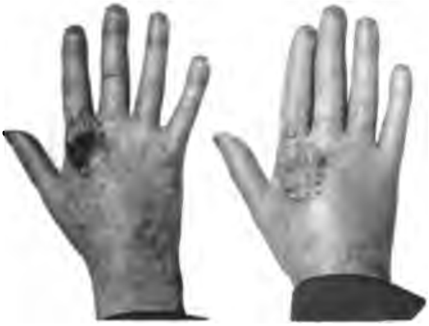
FIG. 19. — W. J. D. Case XIX. March, 1909. Present condition after thirty-four operations.

FIG. 20. — X-ray same case, March, 1909, showing no osteoporosis, in spite of long duration of disease.

FIG. 21. — Photomicrograph, Case XIX., showing in center, nerve surrounded by carcinoma, causing intense pain.

FIG. 22. — H. G. Case XVII. Hand one year after operation. Graft is outlined; note thickness of skin, keratoses and characteristic nail changes.

FIG. 23. — Case XVII. Recurrent growth on forehead, low power. Question of sarcoma or carcinoma. See pathological description.



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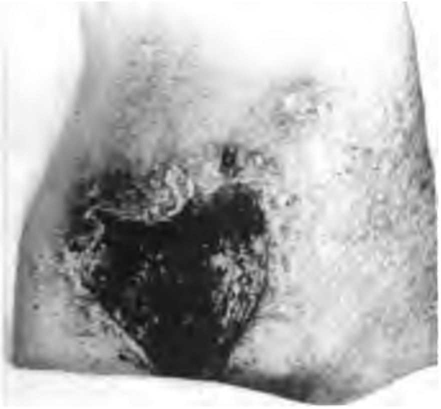

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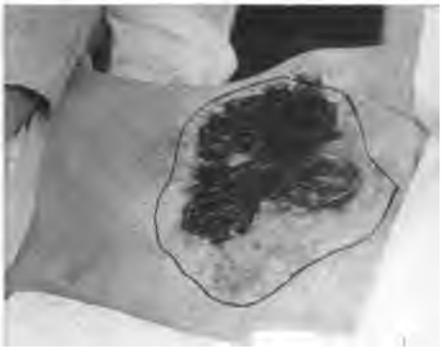

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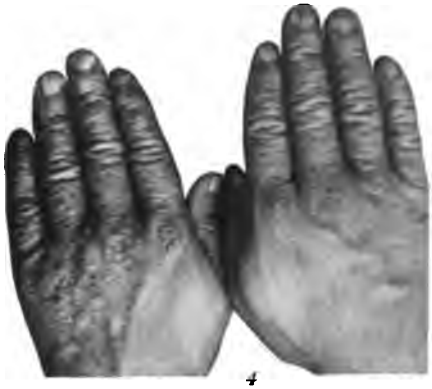

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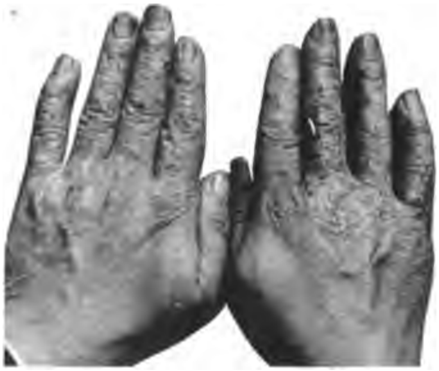

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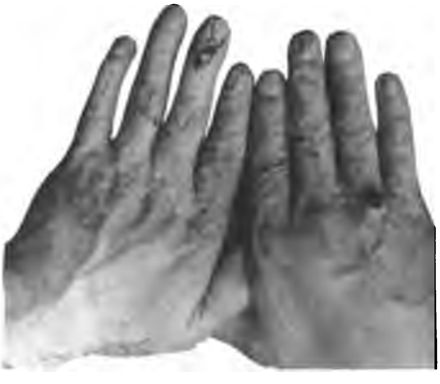

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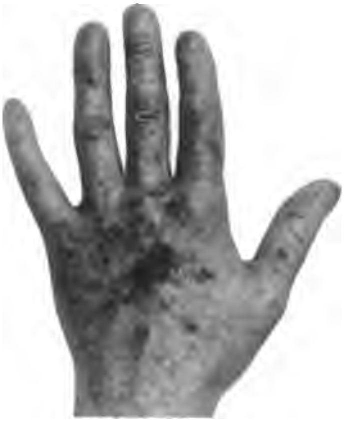

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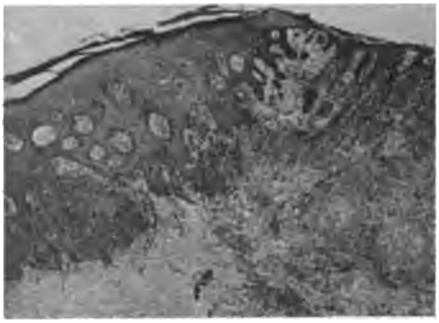

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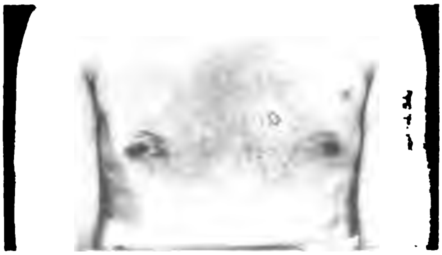

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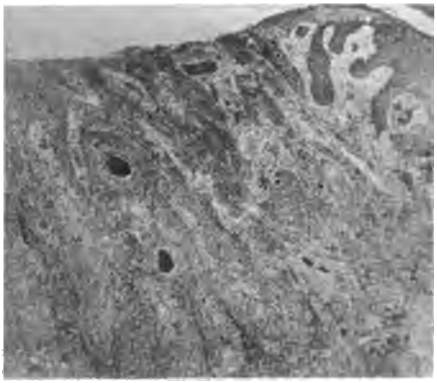

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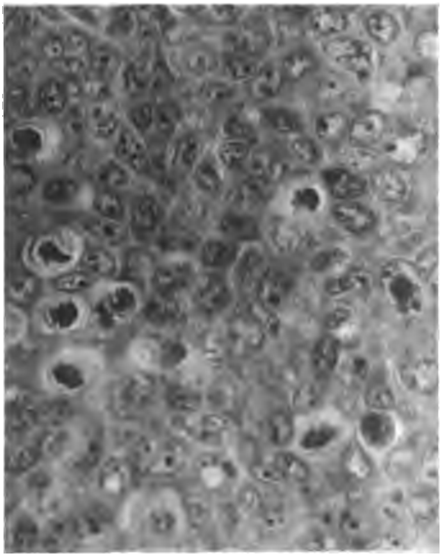

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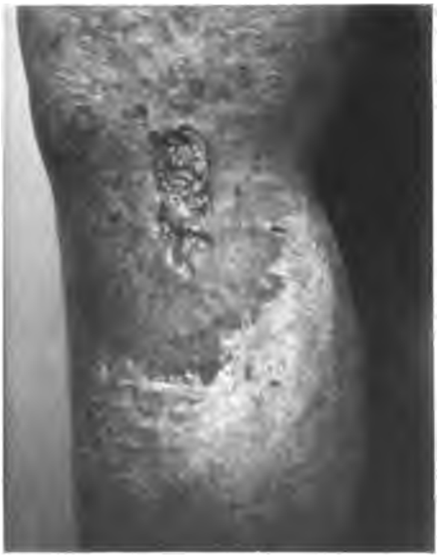

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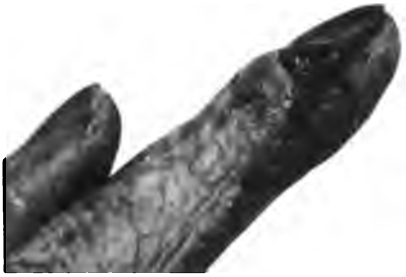
C. A. Porter.

X ray lesions.



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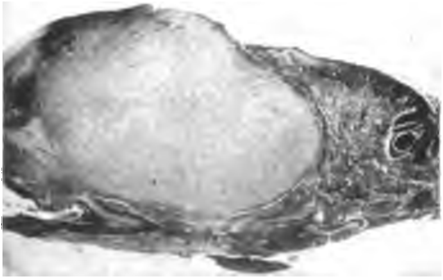

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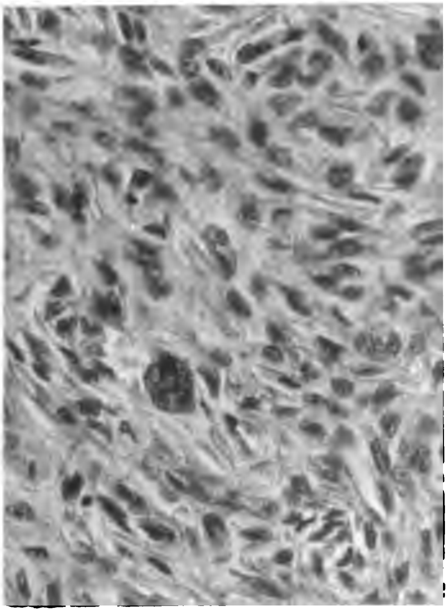

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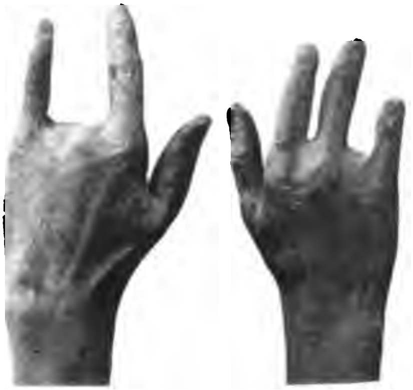

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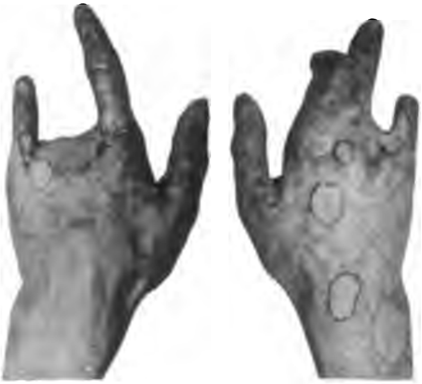

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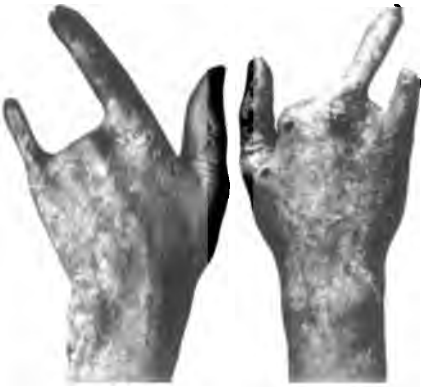

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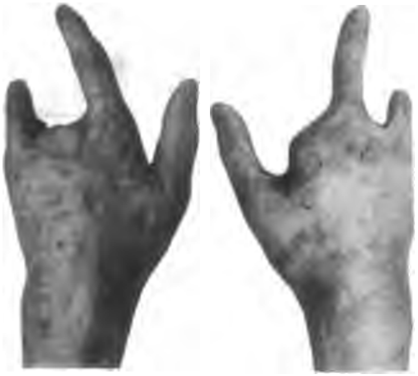

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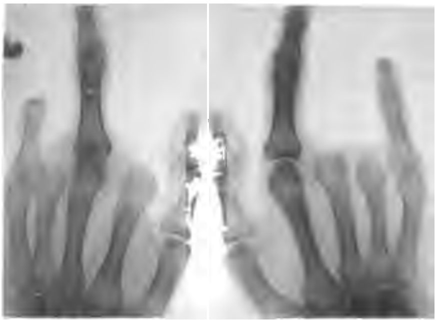

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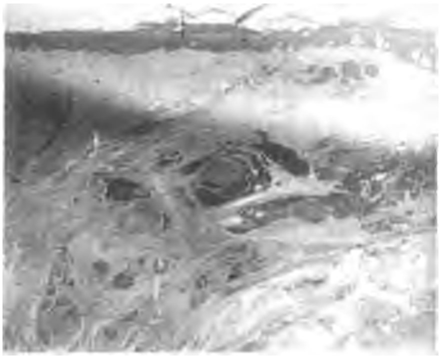

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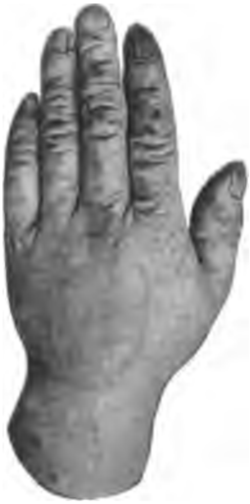

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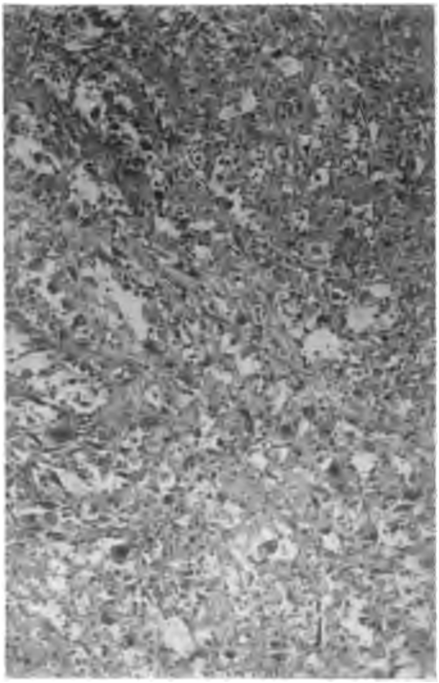

23

THE PATHOLOGICAL HISTOLOGY OF CHRONIC X-RAY DERMATITIS AND EARLY X-RAY CARCINOMA.*

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The development of multiple carcinomata in the skin of patients and operators who have suffered repeated injuries from exposures to the X-rays has occurred so many times that the causal relationship has been generally accepted in all countries. The tumors have made their appearance in each instance reported, several years after the establishment of severe chronic changes in the skin, characterized by great thickening, telangiectases, ulceration, hyperkeratosis, and loss of the epidermal appendages.

The tumors which have developed in the permanently altered skin have been epidermoid carcinomata almost invariably, and microscopically cannot be distinguished from similar carcinomata of ordinary occurrence.

Striking features of the carcinomata following X-ray injuries, already brought forth in numerous publications, are the occurrence in young individuals and the malignant nature of the tumors as shown by the high mortality. The article of Dr. C. A. Porter in this issue describes the clinical features of these cases and presents a summary of those from this country.

The opportunity to make a careful histological study of early X-ray carcinomata and of skin from cases showing varying degrees of the chronic changes which precede the occurrence of these tumors has been a great privilege for which I am indebted to Dr. C. A. Porter. His interest and care has also made it possible to obtain tissues fixed immediately after removal from the patient. In every instance a general anesthetic was employed so that the tissues obtained were not injured by local injections.

All of the material studied for this report was fixed in

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Zenker's fixative, and in every instance immediately after operation. The staining methods employed were the methylene blue and eosine stain, Mallory's phosphotungstic acid hematein, Mallory's connective tissue stain and Verhoeff's elastic tissue stain (all are described in the Pathological Technique of Mallory and Wright, fourth edition).

The descriptions were made chiefly from sections stained by the phosphotungstic acid hematein stain, though the other stains were used for corroborative evidence when necessary. This stain proved to be especially useful in the study of the collagenous fibrils of connective tissue because it brings out differences in staining properties which are not demonstrable by the other methods or any ordinary stain.

As the use of Mallory's phosphotungstic acid hematein stain has not yet become general a brief account of its staining qualities is desirable. Nuclear material and protoplasm are stained blue, collagenous fibrils are stained brownish red. Epidermal, neuroglia, myoglia, and fibroglia fibrils are stained a deep blue. Elastic fibers are stained either a deep purple or purple with a brownish periphery. In the epidermis the superficial layers of cells stain purplish to brownish red. Most cell inclusions stain brownish.

The advantages of this stain in a histological study of skin lesions are:

I. Differential staining of connective tissue.

II. Differences in staining of normal and degenerated collagenous material.

III. The demonstration of fibrils of all sorts and of elastic fibers. The arrangement and development of epidermal fibrils is of importance in the study of altered function of epidermal cells. Myoglia and fibroglia fibrils assist in the identification of smooth muscle and connective tissue cells.

IV. The ease by which products of cell degeneration are recognized when within the cell.

V. The general excellence of the cell pictures, which is largely due to the deep staining of the protoplasm, an especially valuable feature in the study of connective tissue cells in dense fibrous tissue. Eosinophilous cells may be recognized by the presence of deep blue stained granules. Mast cells are not demonstrated, owing to the fixative necessarily employed.

The effects of X-ray exposures upon human and animal tissues have been incompletely determined. Studies from cases similar to those forming the basis for this paper have been few in number, and although the most important of the constant changes have been described, no single observer has had the opportunity of comparing the cases from a series as large as the present one. Many important changes have been overlooked owing to the employment of inferior staining methods.

CASE I. (Case II., Dr. Porter). — R. M., a man aged 55 years was exposed July 23, 1908, for not more than twenty minutes during a fluoroscopic examination. In three weeks a painful area of erythema the size of a dinner plate appeared in the site of the exposure. The edges of the erythema gradually subsided, though the patient continued to have severe pain. The skin broke down and a superficial ulcer 3 x 2 inches resulted, which resisted treatment and steadily increased in size. On Nov. 18, 1908, the whole of the ulcer was excised with a wide margin of surrounding skin.

The material received for examination consisted of tissue hardened in Zenker's fixative, representing a large ulcer about ten centimeters in diameter surrounded by a border of epidermis one to one and a half centimeters in width. The tissue includes the skin and subcutaneous tissues for a depth of one-half to one centimeter.

Description, under the microscope, from sections stained with phosphotungstic acid hematein The most striking lesions, aside from the ulceration, are found in the connective tissue of the corium and subcutaneous tissues. The corium everywhere, beneath the ulceration and where covered with epidermis, is of great density. Immediately beneath the epidermis, however, it has a delicate structure, and is the seat of an active process. The subcutaneous connective tissue exhibits marked degenerative changes, including deposits of fibrin and reparative processes evidenced by the presence of young connective tissue cells.

The deeper layers of the corium are composed of very dense connective tissue, poor in cells, with thick bundles of deeply-staining collagenous fibrils. The latter are stained a deep red and form bundles many times thicker than occurs in the normal corium. The fibrils are coarse in texture and have a more hyaline refractive appearance than those in normal skin. In places there are bundles in which the collagenous fibrils are fused into homogeneous refractive masses. Elastic fibers are very numerous, they run parallel to the collagenous fibrils, in great numbers at the periphery of bundles, but often imbedded within. The elastic fibers vary greatly in thickness, many can be easily resolved only with the highest oil immersion objective (Zeiss 1.5 mm. apo.). All show a central bluish stained core with a brownish outer zone. The connective tissue cells show no abnormalities.

The corium immediately beneath the epidermis for a considerable depth is of loose texture, contains little collagenous material but many young connective tissue cells. The papillæ are absent. These changes are most marked near the ulceration and gradually diminish in the direction of the periphery of the excised tissue. Still every part of the material examined shows absence of papillæ and rarefication of the collagenous material. The collagenous fibrils in this part of the corium are very delicate and stain less deeply than normal, taking a pale brownish color. The individual fibrils or small bundles of them are widely separated. The tissue is infiltrated with polymorphonuclear leucocytes, lymphoid and plasma cells and eosinophiles. There are numerous large, many processed connective tissue cells having extraordinarily large nuclei. There are many dilated capillaries in this rarefied corium, made conspicuous by the large endothelial cells lining them. Migrating endothelial cells are easily found, and there are occasional clusters of them surrounding capillaries. A few capillaries are filled with free endothelial cells, occasionally one contains a single giant cell. In places greatly dilated capillaries lie immediately beneath the epidermis. The epidermis is separated from the corium by a basement membrane, usually well defined, but in places composed of an extremely delicate hyaline brownish stained material. In the connective tissue below the corium are large areas containing deposits of fibrin. The collagenous fibrils are faintly stained and in places changed into a hyaline material. Young connective tissue cells are numerous. There are very few polymorphonuclear leucocytes, lymphoid and plasma cells except about the remains of coil glands. The fat lobules show little change. In places the cell walls are wrinkled and there are accumulations of lymphoid and plasma cells between the fat cells and surrounding capillaries.

The epidermis is uniformly thickened. The horny layer is very thin. The layers of prickle cells and granular cells are of increased thickness. In a few places the epidermis is separated from the basement membrane by spaces filled with finely granular material. The layer of basement or cylindrical cells in general is orderly arranged, but in places there are groups of deeply-stained cells which are irregularly arranged upon the basement membrane. These cells are of the prickle cell type, while elsewhere the basement cells are of the normal type. In these atypical groups the cells are often separated by narrow clear spaces. The prickle fibrils, which are stained a deep blue, run vertically at the base into the basement membrane and here, in places, form a delicate blue line by a series of contiguous arches.

The ulcerated surface is covered with a layer of coarse meshed hyaline fibrin. In most places the fibrin lies directly upon degenerated collagenous material, but there are some large areas of granulation tissue.

The smooth muscle in all sections examined shows very marked changes in the walls of vessels and in the arrector pili muscles. In the latter the cells are of increased thickness, and the myoglia fibrils are of unusual size, though this may be due to the fusion of fibrils. Many of the cells are vacuolated, others are filled with a hyaline brownish stained material. In arteries and veins of the subcutaneous tissues the muscle cells show the same changes as in the arrector pili muscles, though there are many atrophic muscle cells which are surrounded by dense collagenous material of which there is a great increase in the media. In some vessels most of the muscle fibers in a given section are swollen, widely separated by connective tissue and all are filled with masses of hyaline brownish stained material.

Besides the thickening of the media above noted, a few arteries and veins show marked thickening of the intima due to an increase of connective tissue and to swelling and vacuolization of the endothelium. Many arteries and veins are normal in appearance. In the arteries showing the above changes the elastic lamina has disappeared and its place is occupied by a thick dense band of hyaline fibrillary material stained brownish and probably collagenous in nature.

Hair bulbs and sebaceous glands are absent in all parts of this specimen. Certain linear areas filled with inflammatory cells and young connective tissue cells undoubtedly represent the sites once occupied by these structures.

Remains of coil glands are found in all sections. The changes are those of simple atrophy — degeneration with infiltration.

CASE II. (Case III., Dr. Porter). — E. R.; female; 30 years. Was treated with the Röntgen rays for ulcerations on outer side of knee and elbow for about one month during March and April of 1908. Healing of the ulcers followed, then rapid breaking down, ulceration, and severe acute dermatitis. From Aug. 18 until Nov. 7, 1908, there was severe pain from the dermatitis and ulcerations. Examination Dec. 4, 1908, showed characteristic X-ray changes over the inner aspect of the right elbow. The skin was thickened, there were telangiectases and a small ulceration covered with a yellow fibrinous layer. There was marked dermatitis of the left leg from the middle of the thigh to the top of the boot. On the outer side of the knee there was an area six by five inches showing reddened pigmented scars which have healed peripherally, leaving in the center an ulceration three by one inches in extent. Jan. 21, 1901, the ulcer was removed with a surrounding zone of skin one-half inch in diameter.

This tissue was received after hardening in Zenker's fixative. It consisted of an ulcer of the skin three and a half by one and two-tenths centimeters in extent surrounded by a zone of epidermis one to one and a half centimeters in width. The disc of tissue was about one centimeter thick and included the subcutaneous fat.

Description under the microscope. There are the same changes in this specimen that were found in Case I. There are differences of degree, however, that deserve description.

The deeper layers of the corium contain dense collagenous material similar to that in Case I. There are, however, more numerous and larger connective tissue cells throughout. Towards the zone of rarefied corium there is a very cellular layer of connective tissue, the cells of which occur singly or in groups and are separated by wide bands of refractive hyaline collagenous material. The connective tissue cells are large with prominent nuclei and abundant protoplasm having many processes and conspicuous fibroglia fibrils. Elastic fibers are present in extraordinary numbers, in places they run in directions parallel to the connective tissue cells and many are in contact with connective tissue cells.

The connective tissue between the lobules of the subcutaneous fat is dense and similar to that of the corium.

The vessels show much more marked changes than in Case I. There are numerous completely obliterated arteries and all of the vessels in the sections show thickening of the walls with reduction of the caliber of the lumen. The obliteration and thickening of the walls is due to proliferation of connective tissue in the intima and media and to increased thickness of the smooth muscle fibers. In vessels showing lesser changes, the muscle fibers of the media are large, and widely separated by coarse collagenous fibrils and vacuoles. The location of the vacuoles is difficult to determine. Some of them are within young connective tissue cells, which are increased in number particularly in the adventitia. The smooth muscle fibers are swollen, many contain two to four nuclei. The myoglia fibrils are either normal in appearance or more conspicuous than the normal. Where the process is more advanced, the smooth muscle fibers are surrounded by dense fibrous tissue, the myoglia fibrils cannot be demonstrated and the protoplasm has become homogeneous and hyaline in appearance and takes a brownish blue stain.

The thickening of the intima is due to the presence of a dense yet cellular fibrous tissue. In the arteries the elastic lamina is usually totally absent, its place being indicated by a dense band of collagenous material. Occasionally remains of the elastic lamina are found and in these the elastic tissue is broken up into delicate filaments which stain a deep brownish.

The endothelium is often redundant, the cells are large, cuboidal or elongated in shape and often contain large vacuoles. The above changes are most marked in the arteries. In the veins the media shows less marked changes and the thickening of the walls and diminution of caliber of the lumen is due chiefly to a thick concentric layer of large connective tissue cells, with much or little collagenous intercellular substance in the intima.

The corium just below the epidermis shows more marked rarefication and contains more and larger telangiectases than that in Case I. Many very large blood spaces lie directly in contact with the epidermis, separated only by a narrow border of hyaline-changed collagenous material. Others are surrounded by necrotic tissue and are filled with fibrin.

The many capillaries just beneath the epidermis are lined with large endothelial cells, a few are filled with loose endothelial cells. In occasional areas capillaries thus occluded are found, about which all of the connective tissue has undergone hyaline change.

The epidermis is uniformly markedly thickened. In general the relations of the different cell strata are the same as in Case I. But there are many places in the tissue where the lowermost layers of cells present differentiated groups and strata of large deeply-stained cells. In places where the basement membrane is not demonstrable there are irregularities of the bottom layer of cells and individual cells are sometimes found in spaces of the corium, though always connected above with the epidermis Irregular downgrowths between adjacent telangiectases are common, though there is nowhere any evidence of actual invasion or of independent growth, except the doubtful evidence of increased size of the cells and a marked affinity for basic stains. Mitoses are very rare.

Hair bulbs and sebaceous glands are entirely absent. Areas of intense infiltration and active connective tissue multiplication probably represent the former site of these structures. A few atrophic remains of coil glands are found surrounded always by lymphoid and plasma cells.

The ulcerated surface of the tissue is covered for the most part with granulation tissue. In many places dense hyaline fibrous tissue, infiltrated with polymorphonuclear leucocytes, is directly exposed. There is in places slight downgrowth of the epidermis at the edges of the ulcer comparable to that found in cases of chronic ulcer.

CASE III. (Case IV., Dr. Porter). — F. H. S.; male; 40 years. Received Röntgen ray treatment for eczema five years ago. There was marked improvement of the eczema and during that year occasional treatments were given which finally resulted in a severe dermatitis with exfoliation of the skin of both thighs and legs. Four years ago a small ulcer developed on the inner side of the calf and has never healed. Another small ulcer which formed on the thigh healed after a few months. There has been no pain. In August, 1908, the ulceration of the left leg began to spread rapidly, in spite of various forms of treatment including two applications of radium. Examination on Nov. 10, 1908, showed an irregularly-shaped ulcer three by two and a fourth inches. In the center there were occasional islands of growing epidermis, the rest was covered with fibrinous exudate. The skin was undermined and showed no evidence of repair. The whole was excised on Nov. 19, 1908. The tissue received for examination had been hardened in Zenker's fixative. It included the ulcer and subcutaneous fat with a surrounding margin of skin one centimeter in width.

Examination under the microscope. — The deeper layers of the corium are composed of the same dense tissue found in Cases I. and II. In places there are very many young connective tissue cells with conspicuous fibroglia fibrils in the meshes of hyaline-changed collagenous fibers. Some of these connective tissue cells are surrounded by narrow zones of paler staining normal collagenous fibrils. The picture suggests the proliferation of connective tissue and the deposit of fresh collagenous material in the meshes of degenerated fibrous tissue.

The corium immediately beneath the epidermis shows the changes previously described, *i.e.*, the more delicate structure, presence of young connective tissue cells, dilated blood spaces, absence of papillæ and occasional thrombosed telangiectases in contact with the epidermis.

The ulcerated surfaces show active granulation tissue enclosing masses of hyaline changed collagenous material. The granulation tissue arises from a base of very cellular connective tissue in which the connective tissue cells are imbedded in degenerated collagenous material. The epidermis is markedly thickened. The horny layer is almost lacking. The granular layer is narrow, so that the width of the epidermis is composed chiefly of prickle cells. At the edges of the ulcer are downgrowths of the epidermis into the granulation tissue.

The blood vessels show very marked changes. Many large arteries of the corium and subcutaneous tissue are completely obliterated. In these sections it can be shown that the characteristic irregularity of the muscle fibers is largely due to the ingrowth from the adventitia of large fibroblasts, the prominent fibrils of which make it difficult to distinguish these cells from smooth muscle cells. The first suspicion that some of the cells of the media, while apparently smooth muscle, were connective cells was awakened by the finding of branched cells with coarse fibrils. Some of these cells are surrounded by considerable collagenous material and these facts together with the presence of occasional mitoses prove the connective tissue origin of these cells. The vacuoles in the media are probably within degenerated smooth muscle cells. Some small veins and arteries have the media completely replaced by dense collagenous material in which atrophic remains of smooth muscle cells can be found. In all partially or completely obliterated arteries the elastic lamina is replaced by a thick convoluted band of dense collagenous material. In a few large vessels the endothelium of the intima is strikingly swollen and redundant and many of the cells contain large clear vacuoles.

The appendages of the skin are absent. Remains of the arrector pili muscles are found consisting of tracts of young connective tissue enclosing degenerated smooth muscle cells.

CASE IV. (Case VIII., Dr. Porter). — Female; 40 years; S. After an abdominal hysterectomy for a tumor of the uterus was treated with the Röntgen rays for a slowly growing tumor of the abdominal wall which appeared one year after operation. She received one hundred and thirty-six treatments between Jan. 28, 1902, and May 20, 1904. She was well for four years, when characteristic Röntgen ray ulcerations developed, involving the whole lower abdomen. There was necrosis and ulceration of the skin with nodules suggesting small carcinomata. On March 7, 1908, the whole area of skin containing ulcers was excised down to the aponeurosis of the abdominal muscles, where a mass of dense tissue was encountered, supposedly the remains of the tumor for which treatment was given.

Description of gross appearances of excised tissue (by Dr. Thomas Ordway). "The specimen consists of a large irregularly triangular piece of skin and subcutaneous tissue. Its base is 9 centimeters and the sides are 10 centimeters and 11.5 centimeters respectively. It is from 2 to 2.5 centimeters thick except in the central part, where it is .5 centimeter to .7 centimeter thick. The skin surface shows a large irregularly triangular ulcer (5.2 centimeters x 7.5 centimeters x 8 centimeters) conforming in general to the shape of the specimen. The base of the ulcer is depressed from .2 centimeter to .6 centimeter below the rough, irregular, ragged, overhanging and undermined, non-indurated edges. The edges are necrotic, reddish-gray in color, and bathed in bright yellow purulent exudation. The base of the ulcer is bright red to grayish red in color; it is rough and ragged and is irregularly covered with purulent exudate.

"The lower surface of the specimen consists of bright yellow fat tissue through which is a delicate tracery of grayish white fibrous tissue. In the central part of this surface of the specimen (the thinner area referred to above) there is an area (3.5 centimeters x 4 centimeters) of firm, dense, grayish white, glistening, elastic fibrous tissue which is found to be, in many places, in continuity with the base of the ulcer, in other places it is connected by fine and coarse fibrous strands.

"The margin of the skin surrounding the ulcer is from 1 to 3 centimeters in width. It is thicker and firmer than normal skin; it is diffusely reddened with a deep punctate erythema except for numerous slightly elevated, rounded, whitened areas (.3 centimeter to .6 centimeter in size) which have a distinct nodular, almost 'shotty,' feel. On section these nodules are .4 centimeter to .6 centimeter in thickness. The surrounding skin is .2 centimeter to .3 centimeter thick. The bright punctate appearance in the diffuse erythema of the skin surrounding the ulcer is apparently due to marked injection of very small blood vessels."

Microscopic examination: I. Disc of fibrous tissue below floor of ulcer. The tissue consists chiefly of dense fibrous tissue, the bundles of which run in all directions. There are groups of bundles where the collagenous fibrils are fused into hyaline masses and regions where the connective tissue is very cellular and edematous in appearance. The latter areas surround thin walled vessels which often are immediately surrounded by fibrin. Many arteries and capillaries are surrounded by masses of lymphoid and plasma cells with occasional eosinophiles. The amount of elastic tissue is small, and that present is in small masses composed of swollen tortuous poorly-staining clumps.

II. Sections of skin surrounding the ulcer. The corium is composed of dense fibrous tissue rich in elastic fibers and is in general similar to the tissues already described. There is, however, no hyaline change of the collagenous material, and there are no areas containing many young connective tissue cells. The corium immediately beneath the epidermis shows remains of the papillary structure, but shows the same rarefication and other characteristics noted in the other cases. There are numerous large connective tissue cells in this part of the corium with abundant spongy protoplasm and numerous coarse fibrils (Foam cells of Unna). Particularly striking is the occurrence of a layer of homogeneous hyaline changed collagenous material separating the epidermis from the cellular loose connective tissue below. In places this hyaline layer contains migrating polymorphonuclear leucocytes, and occasionally there are groups of a few small, widely separated epidermal cells occupying spaces in this layer, surrounded and infiltrated with leucocytes.

The epidermis is much increased in thickness, chiefly due to the great depth of the prickle cells. Mitotic figures are common in the basement row of cells. Some of the large subcutaneous arteries show eccentric thickenings of the walls.

None of the sections show any trace of hair follicles or glands. An occasional remains of the arrector pili muscles is found in wnich the muscle fibers are small, vacuolated and hyaline degenerated. The fibrils are usually demonstrable.

III. Sections of the elevated areas of skin or nodules found in the skin surrounding the ulcer. The elevation of these areas is due to a great increase in dense fibrous tissue in the corium, which extends down as a continuous layer to the subcutaneous fat. Laterally it gradually merges into the corium of the surrounding skin; there is no line of demarcation. The bundles of collagenous fibers in the nodules and surrounding skin are very dense and hyaline in appearance. The number of elastic fibers is enormously increased. There are no papillæ so that the epidermis lies evenly upon dense hyaline collagenous material or in places upon rarefied connective tissue; in the latter case there is a line of hyaline collagenous material without nuclei immediately beneath the epidermis. There are numerous telangiectases beneath the epidermis and in the dense corium are large blood spaces lined only with endothelium. The skin surrounding the nodules shows marked rarefication of the upper layer of the corium which contains many large telangiectases, some of them filled with fibrin; most of them are surrounded by zones of hyaline collagenous material continuous with the layer beneath the epidermis.

The epidermis in places is greatly thickened and there are processes extending down between the dilated blood spaces or telangiectases. Some of these processes are thick and branched and contain typical epithelial pearls (Figs. 13 and 14). They are always surrounded by zones of lymphoid and plasma cells and an occasional eosinophilic cell. There are numerous mitoses present in these processes and in the adjacent epidermis.

IV. The ulcer and adjacent skin. The floor of the ulcer consists of granulation tissue covered with fibrin and purulent exudate. In numerous places the ulceration extends nearly to the subcutaneous fat. The granulation tissue includes islands of degenerated collagenous material. In places there is no granulation tissue and degenerated collagenous material, infiltrated with leucocytes, forms the floor of the ulcer.

The corium adjacent to the ulcer shows the changes described in the sections from the other parts of this tissue; hyaline change of the collagenous fibrils, and a diffuse appearance of new connective cells between the collagenous bundles without the formation of granulation tissue. Beneath the epidermis there are the same changes observed in the other sections. Near the edge of the ulcers, however, there are many large telangiectases some of which are filled with fibrin (Figs. 4 and 5). In places the fibrin extends through the walls of the vessel into the surrounding tissue. In a few places where a dilated thrombosed vessel is surrounded by epidermis there are masses of epidermal cells extending into the fibrin (Figs. 8 and 9). These cells form tortuous cords and irregular masses of cells sometimes nearly completely filling the vessel. In other places compact groups of cells from the epidermis extend for considerable distances into the corium, and the relations of these epidermal processes is such as to make it certain that extension has taken place into and along the course of one of the dilated vessels. These cell processes show epithelial pearls and many mitoses. Occasionally, in such a process a group of epithelial cells is found which stains more deeply than the surrounding cells, and the individual cells show great irregularity in shape with distorted fibrils, many of which are intracellular (Fig. 14). Here and there in the basal layer are groups of epidermal cells which contain large irregular nuclei often with budding processes containing chromatin material. There are also cells containing two or more nuclei. The cells of these groups are closely packed and are, roughly, concentrically arranged, individually the cells are approximately spindle shaped. Numerous mitoses are also found in these small groups of cells and the whole picture suggests that these groups represent isolated, independently-growing portions of the epidermis.

Many large arteries and veins of the subcutaneous tissue and corium are completely obliterated chiefly through the multiplication of connective tissue cells of the intima (Figs. 18, 21, and 22). In some instances the muscle cells of the media are largely replaced by leucocytes and fibroblasts (Fig. 22). In general the obliterated vessels show the same characteristics as those described in preceding cases.

CASE V. (Case XV., Dr. Porter).—Male; 40 years; a maker of Röntgen ray tubes since 1897. Has had several acute attacks of "eczema." The first ulceration commenced in 1905 on the left hand between the knuckles of the first and second fingers. This has healed and broken down several times. At the time of operation, in April, 1908, there were several keratoses and small ulcerations on the hands. On the whole there has been very little pain associated with any of the lesions. The tissues excised were an ulcer from the back of the left hand, measuring about two by one centimeters;

a horny keratosis from between the knuckles of the index and middle fingers of the left hand, a soft wart-like growth from the nose one-half centimeter in diameter capped with a layer of horny epithelium and a piece of tissue from the chest which came from the center of a thickened pigmented area of the skin showing prominent telangiectases (the chest has been frequently exposed to the rays in making fluoroscopic examinations). This piece of tissue from the chest had a scaly surface with minute elevated scar-like areas. The excision included all the tissues of the skin and subcutaneous fat.

Microscopic examination: I. Keratosis from the back of the hand (Fig. 16). The greatly thickened epidermis has long processes extending into the corium. Between the processes of epidermis are papillæ of the corium, usually long and slender, but occasionally short and thick and containing dilated blood spaces filled with fibrin and leucocytes. The collagenous material of the papillæ, as elsewhere in the corium immediately beneath the epidermis, shows the hyaline change and rarefication noted in the other cases. The vessels in the papillæ are of large caliber and many are surrounded by areas of faintly stained collagenous material in which are strands of fibrin and young connective tissue cells. Collections of plasma cells are common in the neighborhood of capillaries and at the tips of the epithelial processes. The cell masses also contain numerous mononuclear eosinophiles and lymphoid cells and occasionally polymorphonuclear leucocytes. The deeper part of the corium is of the dense hyaline type, with great numbers of elastic fibers, already described.

None of the sections include the large vessels of the deep corium and subcutaneous tissues.

The epithelial processes of the epidermis consist of prickle cells with an abrupt change towards the surface into cells of the type of the stratum lucidum (Fig. 19). Cells of the granular layer type are almost entirely absent. The horny layer is very thick and consists of fused elements in which cell and nuclear outlines can often be distinguished (acanthosis). In places there are spaces in the horny layer containing refractive hyaline transformed cells in which are nuclear remains. The basement layer of cells of the epithelial process is for the greater part regularly arranged and composed of columnar cells. In places the arrangement is irregular, the cells are irregular in shape and widely separated. Such groups are invaded by plasma cells and polymorphonuclear eosinophiles. The epithelial cells show numerous mitoses and in general are small in size and show very prominent prickle fibrils. In places there is growth of epidermis into dilated vessels filled with fibrin which lie in the papillæ or at the base of the keratosis. The epidermis on each side of the keratosis is uniformly thickened and for the most part lies upon a rarefied corium without papillæ. In places there are processes of epidermis extending into the corium, usually around or between telangiectases. The cells of such processes consist of irregularly shaped many processed prickle cells with no definite arrangement. Mitoses are common. These groups of cells are invaded with plasma cells and eosinophile cells and in general present an atypical arrangement and relationship to the surrounding densely infiltrated corium. In addition to such processes there are long stretches of epidermis where the deeper layers consist of similar cells with similar atypical arrangement.

Keratosis from tip of nose (Fig 17). The gross structure is similar to the lesion from the hand. The changes in the corium are also similar. The epidermis is strikingly different because the entire depth of the epithelial processes are composed of a uniform type of cell; large cells with great numbers of mitoses. There are three or four mitoses to every oil immersion field even immediately beneath the layer of horny cells, in some fields there are eight to ten mitoses (Fig. 23). Prickle fibrils can occasionally be demonstrated; usually they are absent. There is a thin layer of horny epithelium which is in many places detached from the active cells below.

At the base of the epithelial processes there are irregular offshoots extending into the corium, always surrounded by densely packed zones of lymphoid and plasma cells and eosinophiles. The epithelial cells composing these offshoots are irregularly arranged, and show absence of or atypical arrangement of prickle fibrils.

In this lesion the picture indicates that differentiation of the epidermal cells into keratohyaline and keratin has ceased and that the whole depth of cells is in active growth with beginning invasion at the base.

Large ulcer from hand. The ulcerated surface is the exposed surface of a vascular new growth of epidermis, atypical in arrangement and containing many mitoses and whorls of keratinized cells. This tissue is microscopically a typical epidermoid carcinoma, and there is invasion of the corium by slender processes of cells on all sides.

The skin on each side of the carcinoma shows the same changes that have been described in the preceding cases, *i.e.*, rarefication of the corium beneath the epidermis, atypical downgrowths of epidermis, vascular changes and hyaline change of the collagenous fibrils in the deep layers of the corium.

Tissue from the chest. This consists of a semi-circular piece of tissue the convex border of which is covered by epidermis, and measures fourteen millimeters long. On each side of the center of the epidermis is an area of new growth very similar to that from the ulcer of the hand. These areas are three to four millimeters long and consist of a vascular new growth of epithelium with whorls of keratinized cells and many mitoses. There is no definite covering of epidermis and the surface is covered with a few small patches of horny epithelium and fibrin. The corium surrounding these two areas is infiltrated in every direction. The whole picture is typical of epidermoid carcinoma. The epidermis between these two areas and outside of them is thick with large processes. The corium and vessels show the same changes that have been described above. In addition to the two areas of carcinoma there is one small area of marked downgrowth of the epidermis, the processes of which are surrounded by masses of lymphoid and plasma cells (Fig. 15). From one of these processes there is a large mass of cells, containing many mitoses, extending into the corium (Fig. 20). Smaller masses project from two adjacent epidermal processes. All are surrounded by densely packed zones of lymphoid and plasma cells. It is evident that the process here is the beginning of a growth similar to the larger ones in the same piece of tissue. A series of twenty-seven sections through this region excluded the possibility of this appearance being due to a tangential section of a larger growth.

CASE VI. (W. W. G., Case III., Dr. Porter). — Male; 41 years. Began work with the X-rays in 1897. In 1900 he suffered from X-ray dermatitis on the dorsum of the right hand, which after healing left the skin dry and reddened. In 1901 the nail of the index finger was removed because of severe pain. In 1903 the characteristic telangiectases and keratoses developed. Persistent painful ulceration necessitated the amputation of several fingers and numerous areas of skin between the years 1903 and 1908. In August, 1908, the small lesion was excised from which the following description is taken.

The material consisted of a circular piece of skin eight millimeters in diameter. The center is covered with a thick layer of horny epidermis, forming an elevated area five millimeters in diameter.

Microscopic examination. The corium shows marked hyaline change of the collagenous fibrils and a great increase in elastic fibers. There are few connective tissue cells to be found except immediately beneath the epidermis where the corium is less dense in structure and where there are minute foci of necrosis and infiltration. Small arteries show thickening of the intima due to a layer of dense collagenous material between the media and endothelium. There are a few normal coil glands but many showing atrophy. The very few hair bulbs and shafts which are in the sections are normal.

The corium immediately beneath the epidermis shows traces only of the papillæ in the form of very slight slender elevations extending into the epidermis. These papillæ contain each a single large capillary with prominent endothelium. The epidermis is thick and forms an even layer except where interrupted by the small papillæ. In many places, however, there are large capillaries running high up into the epidermis, surrounded by a thin zone of refractive hyaline collagenous material. The thickness of the epidermis is chiefly due to increase in the prickle cell layer. The stratum lucidum is of increased thickness. The granular layer is not demonstrable. The horny layer is very thick and consists of a fused mass of incompletely keratinized cells in which the nuclei are large and easily visible (acanthosis). In places where the corium contains areas of fragmented collagenous material and masses of lymphoid and plasma cells, there are shallow downgrowths of the epidermis. The cells of these downgrowths extend laterally between bundles of hyaline collagenous material.

In addition to the above cases, sections prepared at the Massachusetts General Hospital of a case reported by Dr. C. A. Porter (Annals of Surgery, November, 1907) have been studied (Case XIX., Dr. Porter's paper, this issue). The sections were described for the above report by Dr. C. J. White, who paid particular attention to changes in the epidermis.

The series of sections include thirty lesions removed in eight or nine different operations.

All of the changes described in detail from the tissues of the six cases reported above have been found in the tissues from this one individual. This man, according to Dr. Porter and corroborated by the sections, had ten different epidermoid carcinomata removed in five years.

This case, therefore, furnishes strong evidence that the changes described in the epidermis are progressive and develop into carcinomata.

SUMMARY OF THE HISTOLOGICAL CHANGES.

The changes that have been found in the cases described can be summarized best under separate headings as follows: connective tissue, smooth muscle, blood vessels, and epidermis with its appendages.

1. *Changes in the connective tissue of the corium and subcutaneous tissue.* — The most conspicuous of the constant changes in the corium are the rarefication immediately beneath the epidermis and the great density of the connective tissue deeper down (Figs. 1 to 6). The loose textured connective tissue immediately below the epidermis is best interpreted as imperfect repair of degenerated connective tissue due at first to the direct action of the X-rays. The imperfect repair and subsequent degenerations are due most probably to the vascular lesions. The presence of degenerated hyaline collagenous material throughout the depth of the corium must be a direct effect of the rays. That this modified collagenous material represents inert or dead tissue, if such a term may be used in speaking of intercellular substance, is proved not only by the physical appearance and staining reactions, but also by the presence of young connective tissue cells surrounded by normal appearing collagenous fibrils between the masses of this dense collagenous material (Figs. 10 and 11). Further proof is furnished by the finding of isolated masses of similar material, identical in appearance and staining, in the granulation tissue below the ulcerations. This diffuse aseptic necrosis of connective tissue and resulting diffuse proliferation of connective tissue cells is probably directly responsible for the obliteration of blood and lymph vessels. Repeated exposures to the X-rays is in this way accountable for the production of successive deposits of collagenous material, and this is the only satisfactory explanation of the great density of the deep corium. Areas of fibrin in tissues many months after the last exposure to the rays prove that the lesions are slowly progressive. The

marked increase of elastic tissue almost constantly found is difficult to account for. That a new formation occurs, may be proved by the association of delicate elastic fibers with new connective tissue cells. Coarse fibrils present may represent remains from successive crops of connective tissue which have undergone degeneration. The presence of many degenerated fibers suggests this explanation. The question needs more elaborate study for its solution than the scope of this paper will permit.

Many of the connective tissue cells in the rarefied corium are of extraordinary size with large processed nuclei (Fig. 7). Many have numerous small nuclei each containing a particle of chromatin. These cells may be distinguished from endothelial cells by the presence of fibroglia fibrils. Apparently they do not form collagenous material. With the methylene blue and eosin stain the protoplasm stains a deep blue. A few are vacuolated. Similar large cells are found in the fat lobules of the subcutaneous tissue, where the fat is undergoing resorption. The interpretation is that these cells are the result of proliferation under conditions of poor nutrition. As they are found only where there is multiplication of connective tissue cells, they must represent imperfect growth and differentiation.

2. *Changes in the smooth muscle.* — The large size of the arrector pili muscle and the thickness of the nuclei of arteries has led some authors (Unna and Wyss) to speak of the condition as one of smooth muscle hypertrophy. In the present study degenerative changes were always present and the large size of the smooth muscle cells is due to vacuolization (noted by numerous authors) and distension of the cell with hyaline material, the latter easily demonstrable by the phosphotungstic acid hematein stain. In cases of long duration only atrophic remains of the arrector pili muscles were found, showing that the changes noted are unquestionably degenerative. This degeneration of smooth muscle will be considered again in connection with vessel changes.

3. *Changes in the vessels.* — The study of lymphatics in skin, which is the seat of chronic changes, presents

extraordinary difficulties when attempted by the methods employed in this study. The unquestionable obliteration of blood capillaries and the extreme difficulty of demonstrating lymphatics in the tissues studied makes the assumption warrantable that there also has been obliteration of lymphatics.

The changes in blood vessels on the other hand are easily demonstrable. The telangiectases apparently develop from preëxisting capillaries, those of the papillæ of the corium (Figs. 2, 3, 4, and 5). Various stages of dilatation of these capillaries can be seen in connection with other changes, in lesions of varying intensities from different cases. The mechanism of their formation cannot be discovered through histological examination, though obliterative changes in the larger vessels and in the deeper anastomosing capillaries must play a part. Another factor to be taken into consideration is possible traction upon the capillary walls exerted by contracting connective tissue. New collagenous material is frequently laid down between the meshes of older degenerated fibrous tissue and undoubtedly must undergo some contraction before the dense stage is reached. Thrombosis of these telangiectases is common and is usually associated with necrosis of the tissue immediately surrounding as well as of the lining endothelium. Obliteration of capillaries by proliferation of endothelium is a fairly constant finding in most of the cases studied.

The obliterative changes in the veins and arteries are manifested chiefly in a great increase of connective tissue beneath the endothelium and in marked thickening of the media. In the arteries there is disappearance of the elastic lamina and a substitution of a thick, irregular band of hyaline collagenous material. The thickened intima is composed of connective tissue with much collagenous intercellular substance. The endothelium is often composed of swollen and vacuolated cells which occasionally form tufts of cells projecting into the lumen.

The thickening of the media is due to an increase of connective tissue. By means of the stains employed it is possible to demonstrate large fibroblasts and abundant collagenous material between the smooth muscle cells. The latter show varying degrees of atrophy and degeneration — hyaline change and vacuolization (Figs. 17, 21, and 22).

The finding of mitotic connective tissue cells in one case (No. III.) four years after the last exposure illustrates the extreme slowness of this process, as well as its progressive character. In advanced cases of obliterative endarteritis the media is wholly replaced by connective tissue with many large branching fibroblasts. In all of the cases studied obliterative changes have been found in some of the vessels, though normal vessels are usually also present.

4. *Changes in the epidermis.* — Except over foci of acute degeneration of the corium, hypertrophy of the epidermis is a constant finding. The hypertrophy in most cases is in the form of a fairly uniform thickening of the epidermis. In other cases there are local more marked hypertrophies taking the form of keratoses and downgrowths (Figs. 13, 14, 15, 16, and 17). In nearly every case there are numerous areas where the proliferation of epidermis seems unquestionably to be due to disappearance of connective tissue in the corium which has not the power to repair itself (Figs. 12, 4, and 5). The downgrowth of epidermis is analogous to the growth of the corneal epithelium following an incised wound of the cornea, or even gaping wounds of the cornea such as may be made by plowing beneath the corneal epithelium with a sharp triangular-shaped needle (Glover's needle).

Extensive downgrowths of epidermis are found, in several cases, having all of the characteristics of carcinoma with and without evidence of invasion. The cases showing metastatic growths have not been included in this report. Growth of epidermis into thrombosed telangiectases is unusual and must be interpreted as indicative of increased powers of growth (Figs. 8 and 9). Generally the epithelial downgrowths are surrounded by zones of dense infiltration with lymphoid and plasma cells, eosinophiles and polymorphonuclear leucocytes. The epidermis where there is evidence of greatest proliferation of the basal layers has a thin horny layer, as if the whole capacity of the epithelium was taxed to preserve its continuity (Fig. 2).

In general the increased thickness of the epidermis and the production of wart-like growths or keratoses and downgrowths is best explained as the result of constant active proliferation called for by the constant production of small defects in the underlying corium. In Case V. the ulcer from the hand shows undoubted signs of malignancy.

Complete absence of hair follicles, sebaceous and coil glands is the rule in cases of long duration. In less marked cases atrophic glandular structures were found. In no case was there evidence of proliferation of any of the skin appendages. Coil glands were often found in regions where there was total absence of hair follicles and sebaceous glands.

In the description of cases minute cell changes have been omitted. The finding in the epidermis of cells with extraordinarily large nuclei with evidences of direct division into several smaller nuclei was of frequent occurrence. Such cells are most often in small downgrowths or in compact masses of cells inserted in the basal layers. This change and others such as irregularity in size, greater affinity for basic stains and confused arrangement of prickle fibrils have been regarded as concomitant with proliferative and degenerative changes. Possibly the finding of such changes in downgrowths and isolated masses of cells has a slight value as evidence of altered function and increased proliferative power.

Discussion. — All of the cases of this series present only late effects of single or repeated X-ray exposures; and it is therefore beyond the scope of this paper to give a complete account of the effects of X-rays upon human skin. The chronic changes described were necessarily preceded by acute changes, which were the immediate result of the X-ray exposures. No complete investigation of the acute changes has as yet been made. As many of the changes described in this paper have been previously described, a short review of the most important papers is included.

Gassmann (Archiv. f. Dermat. u. Syphilis, 1904, Heft I.) described the vessel changes produced in rabbits. He exposed rabbits until ulcers of one month's duration had been produced and found the following changes in the arteries: The muscularis showed a sieve-like meshwork due to vacuolization. The intima was thickened and broken up, the elastica was fragmented and the endothelium was thickened and in places heaped up two or three cells deep. He claims to have demonstrated obliteration of lymphatics through excessive proliferation of endothelium.

Linser (Fortschritte auf dem Gebiete der Röntgenstrahlen No. 8, 1904–5) excised human skin at varying intervals while the patient was receiving treatment for lupus. At the end of four days there were vessels occluded by thrombi. The endothelium was absent in places and in other places swollen and projecting into the lumen. The media was " broken up " and " fissured." There was slight perivascular infiltration with "round cells." At eight days thrombosed vessels were most numerous and the perivascular infiltration was most marked. At sixteen days there was beginning thickening of the intima. The breaking up of the media and invasion by leucocytes was at its maximum. At twenty days there was marked thickening of the intima due to pure connective tissue without elastic fibers. Many vessels were completely obliterated. At the end of thirty days the " inflammatory changes " were absent. The arteries showed typical thickening of the intima with newly formed elastic fibers, giving to the vessels a shrunken, irregular form with polyp-like projections into the lumen. Linser concludes that the epithelium is not primarily affected even after severe exposure. The only changes that he found was an increase of pigment in the Malpighian layer. The hairs and glands remained normal.

Unna (Fortschritte auf dem Gebiete der Röntgenstrahlen No. 8, 1904–5) reported a study of four cases of chronic X-ray dermatitis. He emphasized the latent period, which varied from a few months to two or three years, and in view of the experimental work already done upon animals stated that the etiology of the lesions was unquestionable. The origin of the telangiectasis is discussed at length. Unna says that a deep obliterating endophlebitis would explain the production of the telangiectasis, but that this has not been proved. The arterial obliteration does not explain it. Unna agrees with Baer and Linser (Münch. Med. Wochenschr. No. 23, 1904) in believing that the blood transforms the X-rays into a form of energy that is injurious to the vessel walls. This he says is supported by the fact that tissues rich in blood as granulation tissue, either exposed or covered by epithelium, is more affected by the X-rays than is normal tissue.

The microscopic appearance of the tissues is given in great detail. Great stress is laid upon the density of the connective tissue of the cutis, which is attributed to degenerative changes following edema. In one case he describes atypical growth of epithelium at the edge of an ulcer, which he believed to be a beginning carcinoma. He did not find changes in the larger vessels of the deep cutis and hypoderm. As a possible cause of the telangiectasis he suggests tension of the smooth muscle bundles called into play by the general atrophy of connective tissue and by the disappearance of intercellular substance between the muscle cells. The keratoses are manifestations of the atrophy of the epidermis, analogous to those occurring in senile changes, sailor's carcinoma, and xeroderma pigmentosum. In retrospection he says that the effects of the X-rays are not limited to the vessels, but affect all parts of the skin, and that there is no indication of an especial susceptibility of the epidermis. The epidermis becomes markedly cornified, in part hypertrophic and predisposed to cancer, in part atrophic. The appendages atrophy, first of all the hair follicles and sebaceous glands. In the cutis there is a marked chronic interstitial edema which loosens and rarefies the collagen and leads to atrophy of the elastic fibers. The smooth muscle alone becomes thickened. Unna emphasizes the difficulty of obtaining good sections because of the great density of the cutis.

Schümann's (Archiv. f. Klin. Chirurg. Berlin, 1907, LXXXIV., Heft 3) description of cases differs from that of Unna's only in the statement that there was proliferation of the sweat glands producing tortuous solid chains of cells. He states that the chronic X-ray dermatitis predisposes to carcinoma and points out similarities between it and other conditions followed by carcinoma such as xeroderma pigmentosum, senile changes, and Unna's carcinoma of sailors.

Wyss (Beiträge z. klin. Chir. Tübingen, 1906, XLIV.) argues for the connection between vessel changes and the origin of carcinoma and says that he is the first to insist upon this point. Following Ribbert's ideas that connective tissue changes precede carcinoma of the skin, and bring about isolation of epithelial cells, he comes to the following conclusions: That the carcinoma cell is one isolated from the rest of the body through the gradual withdrawal of nutrition owing to successive obliteration of vessels; that the cells thus gradually acquire greater capacity for securing nutrition and finally become capable of living at the expense of other cells. He claims that similar vascular changes precede many kinds of carcinomata associated with peculiar practises or occupations.

In a subsequent paper upon the origin of carcinoma (Deutsch. Archiv. f. Chirurgie, Leipzig, XCIII., Heft 6), Wyss offers the evidence furnished by the X-ray carcinomata and the results of a study of fifty early carcinomata in support of Ribbert's theories. In all cases he insists that vascular changes are primary.

Lindenhorn (Beiträge zur klin. Chir. Tübingen, LIX., Heft 2) has collected twenty-nine cases of X-ray tumors, most of them carcinoma and very malignant. The two cases which he adds were in lupus patients treated with the X-rays. The vessel changes described by him were limited to the media. In general he corroborates the work of Wyss and others, but states that the condition is essentially that of premature senescence.

Porter and White (Annals of Surgery, November, 1907) contribute the only important paper on the subject from this country. The histological changes are described without attempt at a summary. Particular attention is paid to the fine changes in the epidermis. Obliterative changes of large veins and arteries are noted as well as obliteration of capillaries by proliferation of the endothelium. Very excellent objective descriptions are given of the keratoses and epithelial hypertrophies and of the telangiectases. Several typical epidermoid carcinomata are described as such, in the case presented by Porter.

GENERAL SUMMARY.

The value of this study of cases here reported is largely dependent upon the wide range of time represented after receipt of the injuries and in the varying amounts of exposure to the X-rays received in the different cases. A sequence of changes can be seen which previous studies have not brought out. The immediate effects of the X-rays, of course, are not included, but the early changes can be inferred from the nature of the chronic changes and the possibilities agree with the results obtained by Linser upon human skin and by others upon animals.

The most important fact brought out in the present study is that after sufficient injury has been done complete repair does not take place. There are in every case active processes going on, and when we consider that some of the cases had received the last exposure two to four years before excision, this fact becomes of great importance. Not only are degenerative and reparative changes constantly taking place in the corium, but the occurrence of progressive vascular lesions is proved by the findings in the media of arteries of young fibroblasts and, in one instance, mitosis of connective tissue cells.

All the changes primarily produced by the X-rays are probably degenerative in character. Normal repair is impossible after a certain amount of injury has been done because of the vascular changes. Thus there is constant degeneration of the tissue in foci and constant efforts at repair. The non-vascular rarefied corium beneath the epidermis is an example of imperfect repair. The finding of necrotic foci here and deeper in the corium, many months and even years after the last X-ray exposure, is conclusive proof of the vascular origin of the degenerations. If the focus of degeneration is small, ulceration does not occur. The continuity of the epidermis is preserved through downward proliferation, and thus a deeper stratum of connective tissue is reached better able to maintain nutrition. That proliferative changes in the epidermis are constantly going on in these cases is proved by the character of the cells of the basal layers and the presence of many mitoses. In many instances the growth into such foci of degeneration has been observed, and even into thrombosed telangiectases. Other evidences of impaired nutrition of the skin are the atrophy of the glands and the absorption of fat tissue and replacement by connective tissue.

The large size of the arrector pili muscles is not due to hypertrophy, but to swelling of the cell and fusion of the myoglia fibrils. In cases of greatest duration these muscles are entirely replaced by connective tissue. The same degenerative changes in the smooth muscle of the media of vessels account for the vacuolization and later the replacement of muscle tissue by connective tissue.

The obliteration of small vessels and capillaries probably takes place slowly and continuously owing to the swelling of collagenous material and proliferation of connective tissue. Endothelial proliferation is possibly a primary cause of capillary obliteration. It would also occur in capillaries occluded from other causes. Excessive infiltration of the corium by lymphoid and plasma cells undoubtedly, as Wyss suggests, helps to interfere with nutrition of epithelium by pressure upon capillaries.

Epithelial proliferation is a constant finding over foci of degeneration in the corium. Ulceration occurs only if the degenerated area is too large to be bridged over by the epidermis. It is probable that long continued proliferative processes are responsible for the hypertrophy of the epidermis. Downgrowth of epidermis takes place to fill small gaps in the corium. This property of downgrowth to fill gaps in the epidermis as well as the growth of epidermis into thrombosed telangiectases must be taken as evidence of increased vitality of the epidermis. Some downgrowths, of a size discoverable only with the microscope, show all of the characteristics of epidermoid carcinoma including the presence of whorls of cornified cells and evidence of invasion between bundles of connective tissue. There are larger epithelial downgrowths which are apparently limited in their growth. The depth seems to be determined by the reaching of viable connective tissue. In the same case all degrees of epithelial hypertrophies, downgrowths, and keratoses may occur, and also growths which are histologically carcinoma and which grow slowly and invade normal tissues. Although this series does not include cases in which metastases have occurred, such cases are too well known to admit of any doubt as to the malignancy of the slowly growing invasive tumors such as are described in Case V.

The evolution of X-ray carcinoma from the smaller lesions, scaly patches, and keratoses has been constantly observed in the clinical study of reported cases so that there can be no doubt that the smaller epithelial growths described in this paper are the starting points of the malignant growths and especially is this true in those cases where similar lesions had already developed into ulcerations which microscopically were unquestionable carcinomata. This evidence is of course wholly clinical, but the observers of these cases have been for the greater part prominent physicians who often were caring for their colleagues. Because the subjects of these experimental carcinomata have been men instead of animals and the observers not laboratory workers does not invalidate the evidence. And this evidence is overwhelming that the carcinomata are produced by changes following severe X-ray exposures. As Wyss has already insisted, the X-ray carcinoma is the first experimental carcinoma.

What then gives rise to the carcinoma? Is it the direct action of the X-rays upon the epithelium or is it the result of changes produced in the deeper tissues? All the evidence advanced by Unna and by Wyss and that found in the present study of cases proves that the epidermis is the least susceptible of the tissues of the skin to direct injury by the X-rays. Even if this were not the case the long "latent" period elapsing between the last exposure to the rays and the appearance of proliferative changes in the skin could not be accounted for except by assuming that the repeated action of the rays excites proliferative powers which remain dormant for months to years. But we do know that during this "latent" period the epidermis is subject to changed conditions involving marked alterations in the character of the connective tissue supporting it and presumable marked changes in the nutrition of the cells. And also that constant proliferative changes are necessitated on the part of the epidermis in order to preserve its integrity. We must then conclude that the factors responsible for the acquisition of great powers to proliferate and eventually the properties of malignancy, are those furnished by the changes in blood supply and in the connective tissue. This theory is supported very completely by the microscopic appearances, especially in those instances where we find evidence of active proliferation of epidermis but incomplete or no differentiation into horny epithelium. The finding of growth of epidermis into fibrin thrombi and into areas of dense bloodless collagenous material seems to be evidence of increased powers of proliferation. Such reasoning as the above, based as it is upon microscopical findings and upon observed gross changes in the living, leads to the conclusion that a slow augmentation of the growth power is achieved (attended with a loss of differentiation) that finally results in the ability of the epithelial cells to derive their sustenance at the expense of other living tissues. The fact that the "virulence" or growth power of mouse tumors may be increased by successive transplantations helps to make the idea acceptable that a similar change may occur in normal human epithelium. There must be conditions however which call for, as does the chronic X-ray dermatitis, a continuous series of proliferative processes, which in a sense are auto-inoculations extending over years.

That connective tissue changes precede and lead to the development of malignant epithelial growths is essentially the theory of Ribbert as propounded in his second Beiträge zur Enstehung der Geschwültse. It is essentially the conclusion reached by Goebel (Zeitschr. f. Krebsforschung, Bd. III., Heft 3) in his study of bladder tumors associated with Bitharzia disease. It is the conclusion reached by Wyss in his study of X-ray carcinoma and of early carcinoma in general. But none of these observers has described the mechanism leading to the repeated proliferation of the epidermis. Neither have they furnished microscopic evidence of the various histological changes assumed to take place, and the idea conveyed is that the acquisition of malignant power takes place suddenly.

The acquisition of malignant powers is completed during years of active proliferation accompanied by progressive impairment of nutrition. This much we have microscopic evidence for, and the hypothesis that the former is a direct sequence of the latter seems justifiable.

Finally it is not far removed from von Hansenmann's hypothesis of tumor origin, that of anaplasia wherein the power of growth is attributed to reversion to the stage of proliferative function at the expense of that of differentiation. The only difference between this and the theory suggested above is, that in the latter this power is assumed to be acquired through changed environment.

The apparent demonstration of the origin of the multiple carcinomata of chronic X-ray dermatitis in primary connective tissue changes and the attending disturbances of physical and nutritive conditions does not speak for the acceptance of the old ideas of trauma and irritation as causes for the origin of carcinoma. In the case of X-ray carcinoma we are dealing with injuries incapable of complete repair and therefore progressive in character.

Other peculiar forms of injury associated with carcinoma, such as those occurring in aniline workers and workers in the various products of combustion and distillation of coal, those associated with certain diseases, as syphilis (leukoplakia) and lupus, the Kangri carcinoma of the natives of Kashimir and "horn core" cancer of cattle in India, deserve the most careful study to see if the antecedent processes are similar to those in X-ray cases. There is an apparent analogy in the instances of cancer associated with lupus and syphilis in that in these diseases there are continuous connective tissue changes taking place for years.

Considering the present great activity in cancer research it seems strange that more attention has not been given to this field of work and it is the earnest hope of the writer that this paper may stimulate investigation and experimental work along these lines.

[The writer is greatly indebted to Professor Councilman for the privilege of using the photomicrographic apparatus of the Pathological Department of the Harvard Medical School, where these photographs were made.]

DESCRIPTION OF PLATES.

PLATE XXXIX.

FIG. 1. — Case V. Second operation. Skin from chest showing a recent focus of degeneration in the corium beneath the epidermis. Epidermis remains intact.

FIG. 2. — From Case IV. shows rarefication of the corium immediately beneath the epidermis. The lower strata of cells in the epidermis are large and are stained deeply with the basic stain. The horny layer is imperfect. Rapid multiplication of the epidermis is evidenced by the finding of many mitoses. The large capillaries are probably the precursors of telangiectases.

FIG. 3. — Higher power of Fig. 2 showing the character of the basal layers of epidermal cells and the structure of the corium.

FIGS 4 and 5. — From Case IV. These photographs show many large telangiectases and extreme rarefication of the corium beneath the epidermis. Fibrinous thrombi are forming in several of the telangiectasis. The downgrowth of epidermis in the rarefied corium is well shown. This condition is always accompanied by endarteritis and endophlebitis.

PLATE XL.

FIG. 6. — From Case IV. High power showing structure of rarefied corium and a connective tissue cell in mitosis.

FIG. 7. — From Case IV. Connective tissue cell from rarefied corium. Atypical cells of this type and cells in mitoses are abundant in the corium

beneath the epidermis and furnish evidence of the continuous change which is reparative in nature and secondary to obliteration of vessels.

FIG. 8. — Case IV. Low power photograph of epidermis growing into thrombosed telangiectases.

FIG. 9. — High power detail of Fig. 8.

FIG. 10. — Case I. shows hyaline change of collagenous material with absence of connective tissue cells.

FIG. 11. — Case I. Hyaline degenerated collagenous material in the neighborhood of vessels showing invasion by connective tissue cells and deposit of new collagenous material between the masses of the old.

PLATE XLI.

FIG. 12. — From Case IV. High power photograph of epidermis growing into rarefied corium. At this point the corium is represented by a delicate homogeneous material without cells. The epidermis dips down at this point. The large size and deep staining of the cells is shown. There is a single mitosis.

FIG. 13. — Case IV. High power view of epidermal processes extending into rarefied corium. The process on the left shows a whorl of keratinized cells. At the tip of the process there is marked lymphoid and plasma cell infiltration and the epithelial cells are large and irregular with many mitoses.

FIG. 14. — Case IV. Epidermal process extending into rarefied corium. Evidence of independent growth shown by the arrangement of cells and production of epithelial whorls. In the bar connecting the two larger masses is a nest of cells with atypical arrangement suggesting independent growth.

FIG. 15. — Case V. From chest. Epidermal process extending into corium and having the characteristics of the larger growths which were frankly malignant in character.

FIG. 16. — Case V. Keratosis from back of hand showing activity of cells at the base, but also the presence of the different layers of the epidermis with dense horny layer.

FIG. 17. — Case V. Keratosis from tip of nose showing absence of differentiation. The whole thickness of the epidermal process is composed of large cells of a uniform type. There are many mitoses.

PLATE XLII.

FIG. 18. — Case III. Obliterated artery from the deep corium showing separation of muscle cells by connective tissue. The lumen is filled by connective tissue.

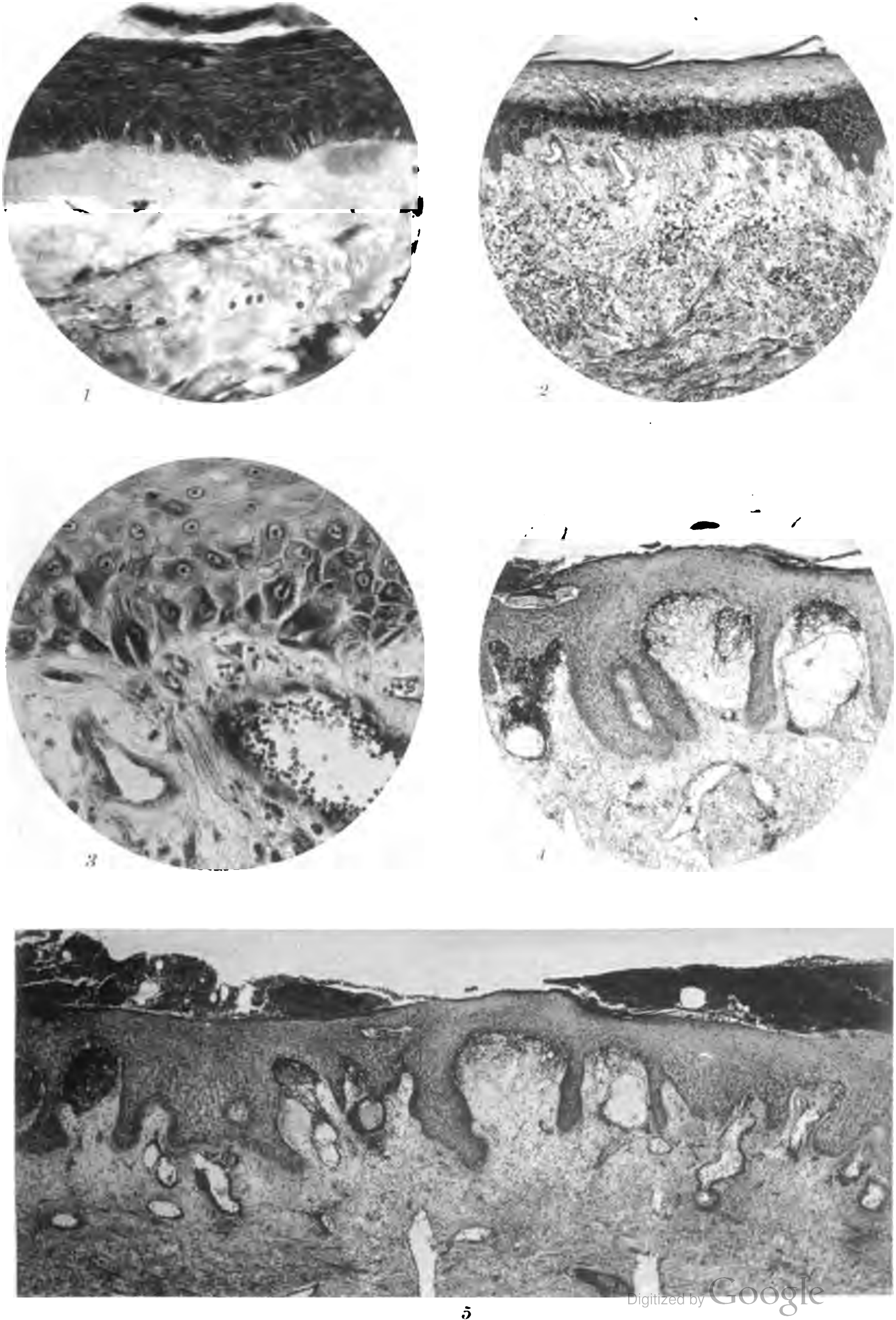
FIG. 19. — High power view of Fig. 16.

FIG. 20. — High power detail from Fig. 15 showing invasion of epidermis into the corium.

FIG. 21. — Low power photograph of an obliterated artery from Case III.

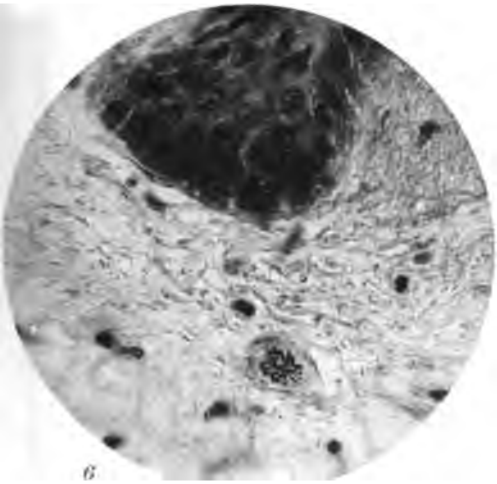
FIG. 22. — Obliterated artery from Case V. At this stage the muscle fibers have disappeared and there is marked infiltration.

FIG. 23. — High power detail of Fig. 17 showing large number of mitoses.



Wolbach.

Chronic X ray dermatitis.



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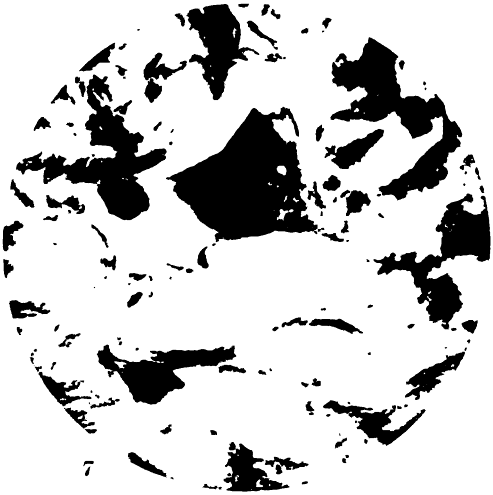

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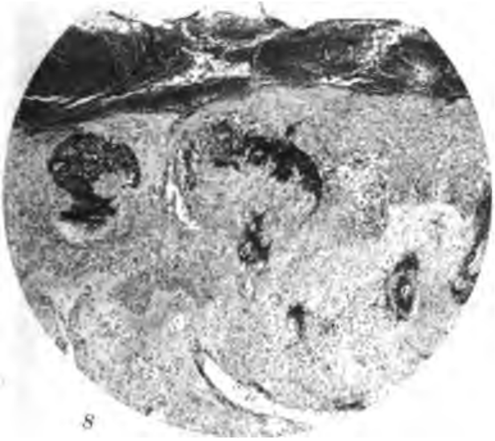

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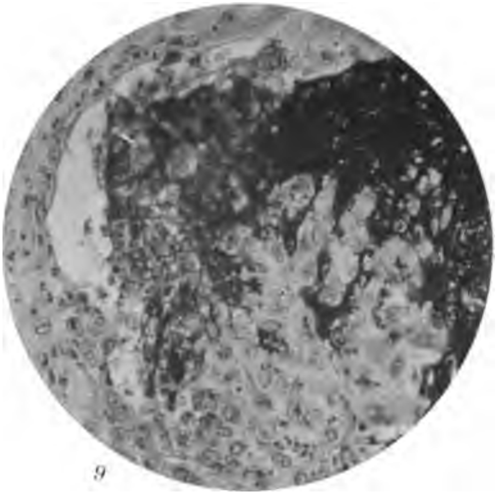

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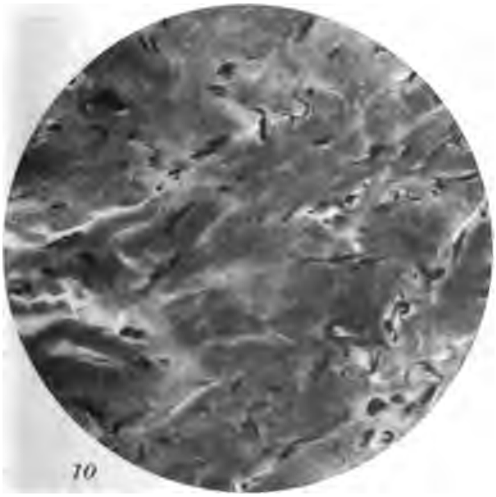

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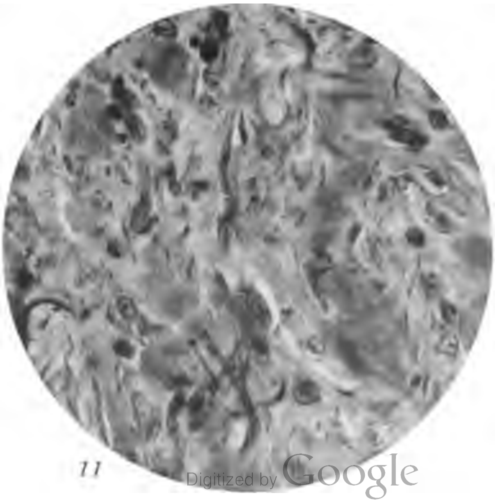

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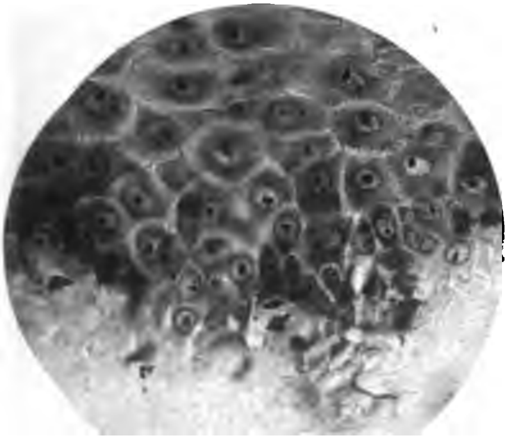

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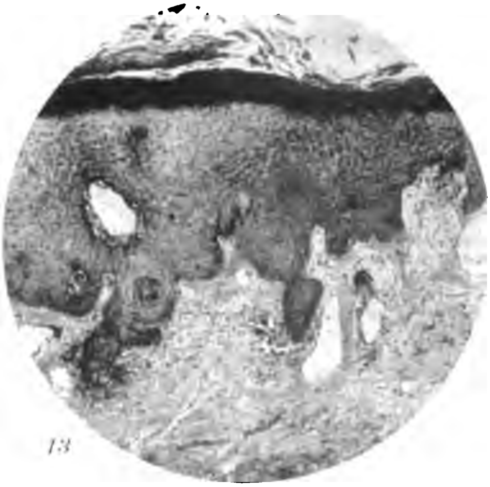

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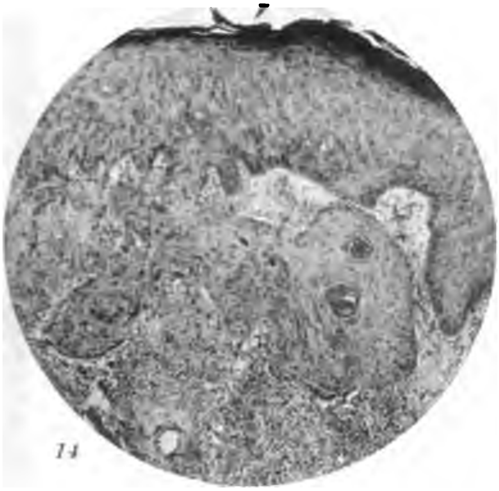

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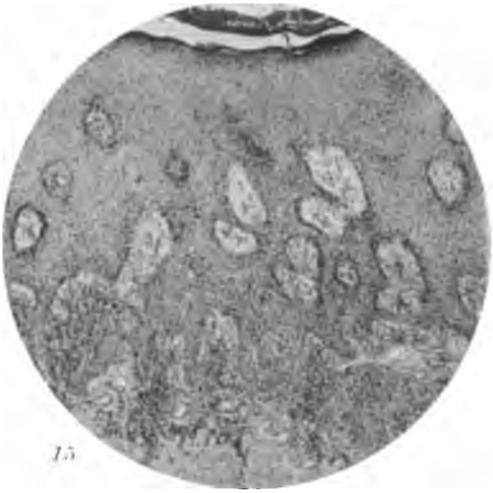

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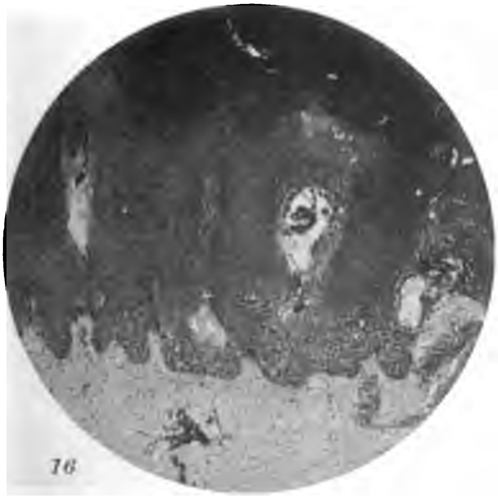

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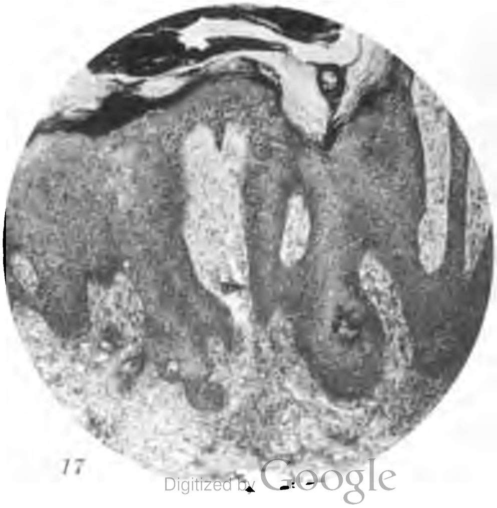

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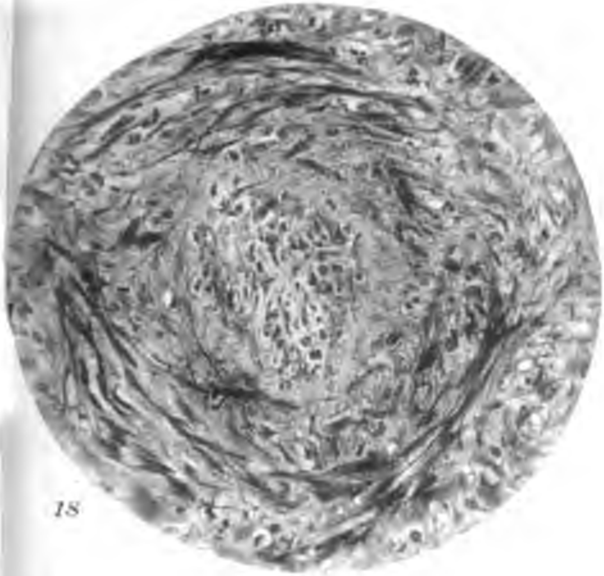
Wolbach.

Chronic X ray dermatitis.



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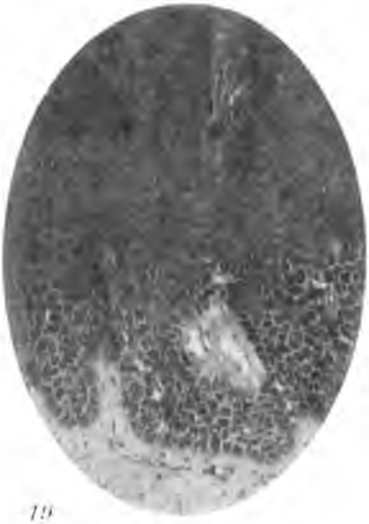

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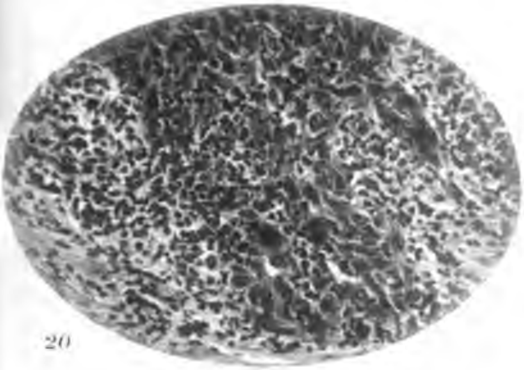

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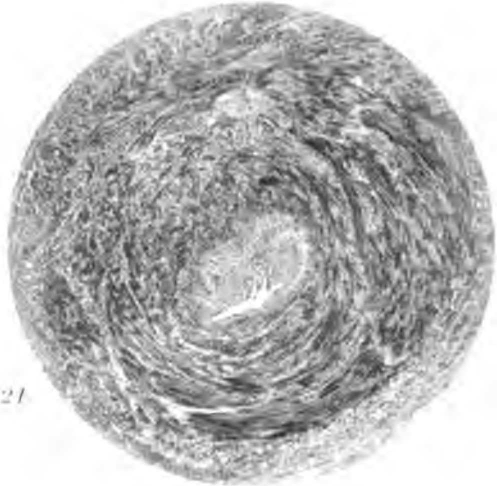

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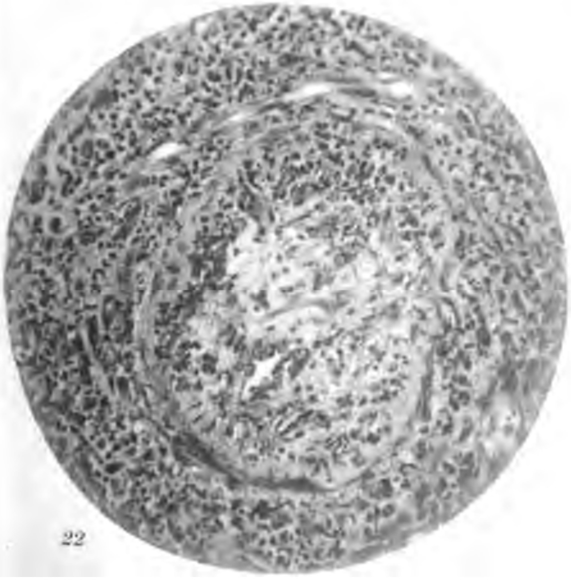

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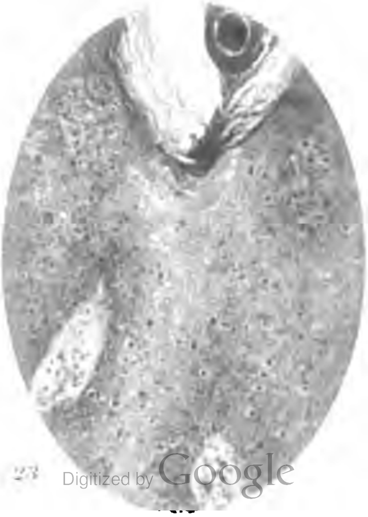

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Chronic X ray dermatitis.

CHRONIC PANCREATITIS WITH TUMOR–LIKE NODULES IN THE CAT.*

THOMAS ORDWAY, M.D.

In order to distinguish and interpret intelligently the pathological processes which may be produced in animals under various experimental conditions such as the action of parasites, toxines, and drugs, and by surgical procedures, it is first necessary to become acquainted with the lesions which occur naturally in the animals used. The frequent occurrence of lesions in apparently healthy animals emphasizes the necessity of using a sufficient number of controls in experimental work.

Dr. E. E. Tyzzer first called my attention to lesions of the pancreas in cats and furnished material from a series of twenty-two autopsies. The purpose of this paper is to record the frequency and describe the character of pancreatic lesions in cats under natural conditions and to consider briefly associated gross lesions found in some of the other organs.

Material and methods. — My data and conclusions are based upon the lesions found in autopsies on forty-four cats, such as are ordinarily used for laboratory experimentation, of various ages and physical conditions. Some of the animals had been the subject of physiological experiments of short duration, others of feeding experiments. These will be specially designated and referred to later.

The cats were killed by ether and at once autopsied completely, head and cord excepted. In all cases portions of organs were fixed in Zenker's fluid and four per cent formaldehyde. Paraffin sections, transverse and frontal, from various portions of the head, middle, and tail of the pancreas were stained by the eosin-methylene-blue method. In certain cases serial sections were made and stained by hematoxylin and eosin. Many of the sections were also stained by

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* Received for publication July 26, 1909.

Mallory's phosphotungstic-acid-hematoxylin and connective tissue methods. In some instances lesions were not present in all the slides from the same pancreas, but when five or six slides from different parts of one pancreas failed to reveal lesions as many as ten or twelve blocks from various portions also showed no lesions.

Lesions of pancreas. — In but few cases could gross lesions of the pancreas be recognized (vide infra); in some cases which showed marked lesions microscopically the pancreas was of normal pinkish color and soft consistency. In others it was small and whitened; still others showed small grayish-white to light yellowish nodules, some barely perceptible, others one to two millimeters in diameter; while in the most marked cases the nodules were as large as five millimeters and scattered generally throughout the pancreas (Fig. 1).

Microscopically the pancreatic lesions vary greatly in degree and form from minute foci of typical chronic inflammation in the interlobular connective tissue, without change in the glands, to great increase in the interlobular and inter-acinal connective tissue, with changes in the glands, and to the formation of cysts of various sizes; indeed, changes so marked as to obscure entirely the normal structure of the pancreas (Figs. 2 and 3).

Though all transitions from the normal pancreas to that with the most marked changes were found, I have, nevertheless, for the purpose of description and analysis, grouped the lesions into more or less arbitrary types. In some cases a single type of lesion occurred, but usually more than one was present in the same pancreas. These types show, I believe, different stages and probably different degrees of one process, evidenced by the varied changes in the gland cells and in the connective tissue between and within the lobules.

TYPES OF CHRONIC PANCREATITIS. — I. Foci of interlobular chronic inflammation. — In the interlobular connective tissue occur focal lesions which vary from small groups of lymphoid cells to larger areas in which there are numerous lymphoid and young connective tissue cells and many eosinophiles. When these lesions are small the glands are normal (Fig. 4). In marked cases, however, the increased connective tissue encroaches upon the ducts and there is an increase in the inter-acinal connective tissue. Therefore, it seems probable that the foci of chronic inflammation in the interlobular connective tissue represents early stages of the more decided lesions.

II. Increased connective tissue between the glands. — This connective tissue increase may be focal or diffuse; it consists of an increase between the separate glands and is usually associated with interlobular chronic inflammation (Type I.). The focal lesions are sharply circumscribed, rounded areas in which the glands are more or less separated by connective tissue. The gland cells occur for the most part in tortuous columns, there is less distinction between the peripheral and central portion of the cells than normally, and the cells take a more homogeneous even stain. Where this connective tissue increase is marked, the gland cells show all degrees of atrophy. About these focal areas there is usually a connective tissue capsule varying from a few strands of connective tissue to a thick capsule containing many eosinophiles. They resemble adenomata. Adjoining lobules appear normal (Fig. 5).

In addition to such circumscribed areas, chronic pancreatitis also occurs as a general increase in connective tissue which separates the glands. It extends diffusely throughout adjoining lobules; there is no limiting capsule, but there is usually an associated increase in the interlobular connective tissue (Fig. 6). The changes in the gland cells are similar to those occurring in the focal nodules of chronic pancreatitis.

III. Glandular hyperplasia. — These lesions occur chiefly in circumscribed nodules and in some cases also closely resemble adenomata (Fig. 7). The structure and arrangement of the acini are very atypical; in some nodules the gland cells appear very numerous, the nuclei are vesicular, the cytoplasm varies greatly in amount and takes with eosin a homogeneous pinkish stain. The peripheral and central portions of the cells are not differentially stained. The cells do not have a gland-like arrangement; they are disposed irregularly. The adenoma-like nodule appears larger than neighboring lobules, the adjacent gland cells of which are flattened and seem to be pressed upon. In other similar foci the cells are swollen and vacuolated as if they had undergone a fatty change. In a few instances diffuse areas of atypical cells were found. Although some of these nodules of glandular hyperplasia with atypical gland cells contain very little connective tissue, yet most of them show a varying amount.

In some of these focal areas the interglandular connective tissue is restricted to portions of the nodule; such areas frequently have a connective tissue capsule limited to a portion of the periphery, while other parts of the same nodule showing little or no connective tissue contain very atypical gland cells and in all respects resemble the adenoma-like areas above described. Hence the same nodules may show both increased intralobular connective tissue (Type II.) and glandular hyperplasia (Type III.). Such changes going hand in hand with chronic pancreatitis apparently form a part of a single process.

IV. Cysts.—Dilatations of the tubules or smaller ducts occur frequently. They vary from small dilatations of the acini to large cystic spaces in the lobules (Fig. 3). Immediately about these cysts the gland cells are somewhat flattened and distorted, and in marked cases there is a great increase in the neighboring interlobular connective tissue which contains numerous lymphoid cells and eosinophiles. In the cysts are numerous similar cells and also large endothelial cells. When cyst formation is at all marked there is a corresponding increase in interlobular and interacinal connective tissue and serial sections show that the ducts from the cystic lobules run out into and are apparently compressed by the increased interlobular connective tissue. In addition to these four definite, though artificial types, a single instance of focal necrosis in an otherwise normal pancreas was noted.

These types of lesion occur separately and in combination; all transitions from the very minute to the most marked were observed. Neither within the focal lesions nor within the diffuse can islands of Langerhans be distinguished. The islands occurring in unaffected portions of the pancreas appear normal in practically all instances. The islands of Langerhans were atypical in one case in respect to number; there were fifteen to eighteen on the average in a low power field; normally but four to six occur. In another case the nuclei of the islets were very abundant, small, and round, resembling lymphoid cells.

Of the forty-four cats autopsied, twenty-nine, or sixty-six per cent, showed microscopic lesions in the pancreas; of these but nine, or thirty-one per cent, showed gross lesions. Of thirteen cats which had been fed a long time with certain food preservatives, six, or forty-six per cent, showed pancreatic changes; of these six, three were slight, two moderate, and one marked.

Of thirty-one supposedly normal control cats, twenty-two, or seventy-one per cent, showed lesions in the pancreas. Of these, eleven were slight, thirteen moderate, and three marked. Thus the control cats showed pancreatic lesions as frequently as the cats fed with the food preservatives.

Foci of chronic inflammation (Type I.) occurred in seventeen out of the forty-four cases examined, or thirty-nine per cent. It was slight in ten cases, moderate in six, and marked in one. Intralobular connective tissue increase (Type II.) was present in sixteen cases, or thirty-six per cent, slight in seven, moderate in eight, and marked in one case. Glandular hyperplasia (Type III.) was found in fourteen cases, or thirty-two per cent, slight in eight, moderate in four, and marked in two cases. Cysts (Type IV.) occurred in nine cases, or twenty per cent, slight in four, moderate in four, and marked in one case.

Of the twenty-nine cats having pancreatic lesions, eleven showed one type, seven two types, nine three types, and two four types of lesion. Certain cases presented lesions of but a single type: foci of chronic inflammation in four cases, intralobular connective tissue increase in three cases, and glandular hyperplasia in four cases. Cysts did not in any case occur without an increase in interlobular or interacinal connective tissue.

The focal areas, Types II. and III., somewhat resemble adenomata from the fact that they are circumscribed nodules of epithelial cells, differing considerably from the surrounding glands.

Against the view that they are adenomata are the facts that in many of such nodules there is a marked increase in the interglandular connective tissue, either general or in part, that all transitions are found from the typical foci of interacinal connective tissue increase, with capsule of interlobular chronic inflammation, to the nodules of glandular hyperplasia. In many of the transitional nodules zymogen granules were present in the gland cells. Indeed a few nodules were found which on one side showed typical glandular hyperplasia and on the other typical focal connective tissue increase, with capsule of chronic inflammatory tissue; between these two the glands showed all transitions from normal to very atypical.

With the increase in the interlobular connective tissue certain of the ducts are encroached upon and cysts of varying size are formed. This view of their origin is favored by the fact that serial sections show ducts from the cystic areas running into and being apparently pressed upon by the newly formed connective tissue. Cellular content similar to that found in the cysts is often found in the ducts; moreover the cysts do not occur without an increase in interlobular and interacinal connective tissue.

The occurrence of these lesions is not appreciably affected by the age, sex, feeding, or general condition of the cats. No bacteria or animal parasites were found associated with the lesions. Dr. E. E. Tyzzer has called my attention to "a very marked case of chronic pancreatitis associated with cholecystitis in a cat, which was apparently brought about by distoma which were present in the pancreatic ducts and very numerous in the liver." He says that the lesions in the pancreas appear to correspond with those described by me; that an important part of the process is stenosis of the ducts with cyst formation. Sections prepared after the Levaditi method did not reveal spirochetes.

Associated lesions. — Although special attention was directed to the pancreas, any gross lesion of the other organs was noted and its character determined by subsequent section and microscopic examination. Such associated gross lesions were found most frequently in the liver, nine cases, and kidneys, twelve cases. In the former cirrhosis occurred in three cases, varying from a slight to a very marked degree; in six cases so marked a fatty change was present that the vacuolization rendered the hepatic cylinders unrecognizable.

In the kidneys there were twelve cases which showed chronic nephritis, varying from a slight to a very marked degree; desquamation of cells within Bowman's capsule occurs and large eosin-staining cells block the lumina of many of the tubules. Within the tubular epithelium and also within the lumina are many deeply-staining bodies, some more or less needle-shaped, others resembling coarse fibrils. In general the most marked kidney lesions occur in the cases in which the pancreas is most affected. Fat was present in practically all the kidneys of the series and was definitely restricted to the convoluted tubules; this, however, is normal.

In two cases enlarged and calcified mesenteric lymph nodes were found; in these cases there were areas of necrosis with slight peripheral reaction of epithelioid and lymphoid cells; no tubercle bacilli were found. There were two cases of severe scabies, sections of the ears of which showed marked keratosis, and moderate general and irregular proliferation of the epidermis. In the keratoses the organisms typical of scabies were present in large numbers.

SUMMARY AND CONCLUSIONS.

1. Pancreatic disease is very frequent in cats under natural conditions; consequently the necessity of sufficient controls in experimental work is indicated.
2. The lesions are of the nature of chronic inflammation.
3. The lesions vary from small foci of chronic inflammation and focal and diffuse increase in interlobular and interacinal connective tissue to nodules of atypical glandular tissue and cysts somewhat resembling adenomata.
4. Lesions of other organs are frequent, particularly in the kidneys and liver.
5. The cause of these varied lesions in the pancreas is as yet not evident. In some cases they may be caused by distoma in the pancreatic ducts.

DESCRIPTION OF PLATES.

Plate XLIII.

Fig. 1. — Portion of duodenum and adjacent pancreas of cat, showing light nodules, two to five millimeters in diameter, scattered generally throughout. x 1.5.

Fig. 2. — Normal pancreas showing islands of Langerhans and a duct of average size. The lobules are somewhat separated. x 60.

Fig. 3. — Cysts with marked increase in interlobular and moderate increase in interacinal connective tissue; also a dilated duct containing material similar to that in the cysts. x 60.

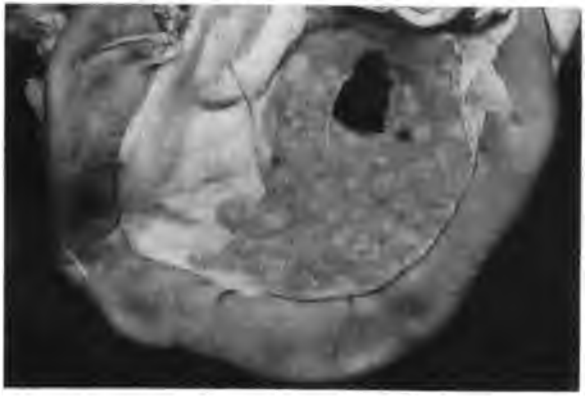
Plate XLIV.

Fig. 4. — Shows a focus of interlobular, chronic inflammation (at the left). The glands elsewhere are normal. x 60.

Fig. 5. — A sharply circumscribed, rounded area in which the gland cells are more or less separated by connective tissue; otherwise the glands are normal. x 60.

Fig. 6. — Diffuse increase in interlobular and interacinal connective tissue generally, with acini in varying degrees of atrophy. Portions of focal nodules at bottom of photograph. x 60.

Fig. 7. — Shows a portion of a large focal nodule of hyperplasia of gland cells resembling an adenoma. x 60.



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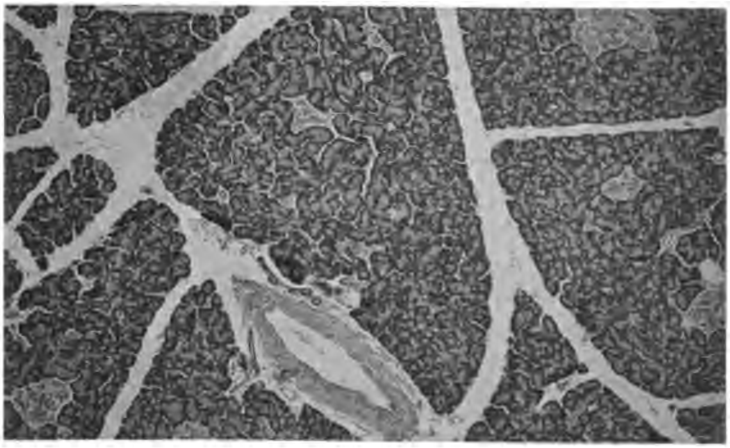

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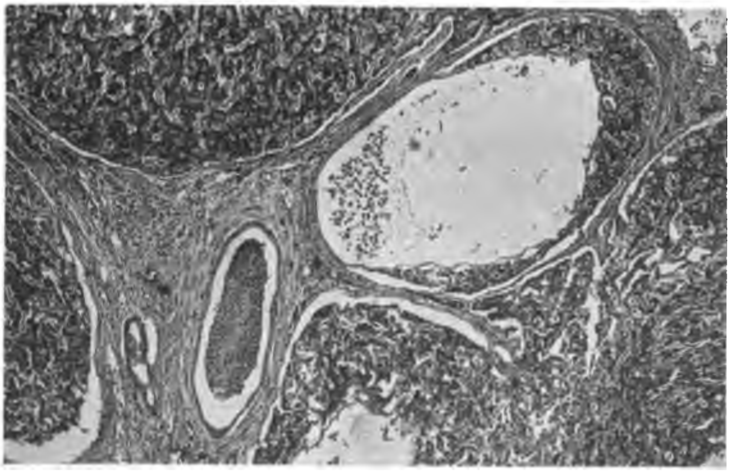

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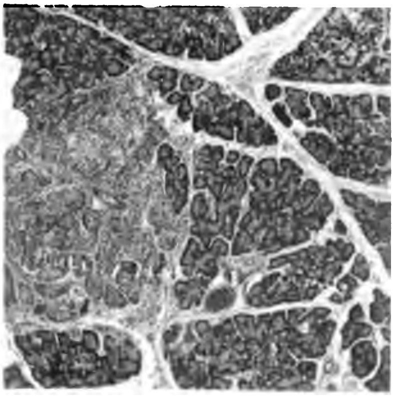

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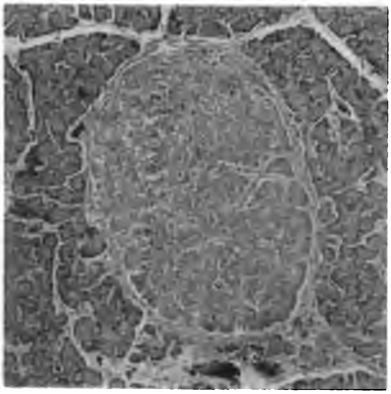

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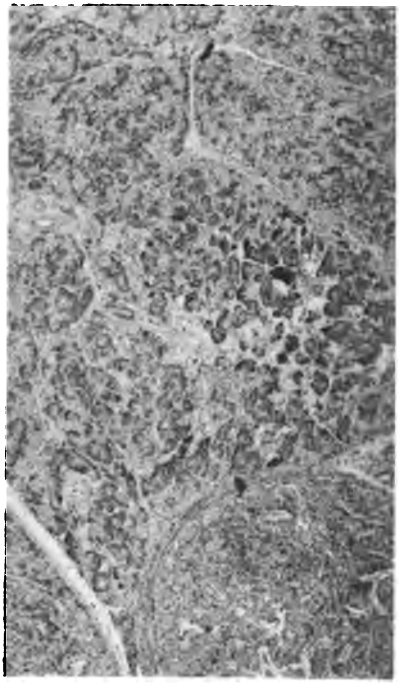

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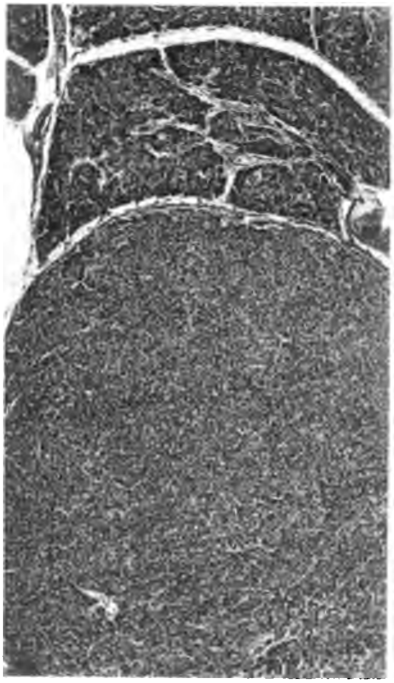

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Ordway.

Pancreatitis in the cat.

TUMORS IN THE COMMON FOWL.*

E. E. TYZZER AND THOMAS ORDWAY.

Since up to the present time comparatively few tumors have been recorded in birds, the following nine cases of tumors in fowls are described somewhat in detail. A histological study was made of each tumor, and in two instances the tumor was inoculated into several normal hens.

Several different types of tumor have already been noted in fowls. A squamous-cell carcinoma of the floor of the mouth of a hen has been recorded by Pick,[1] and a similar tumor also situated in the mouth has been described by Koch.[2] An attempt was made in the latter case to transplant the tumor to other hens with negative result. Koch also describes cysts containing feather follicles, "cystoma pennifera," in hens and geese which he considers analogous to the dermoids of mammals. Ehrenreich[3, 4, 5] showed that tumors are of frequent occurrence in fowls. Seven malignant tumors were found on examining four thousand hens which were brought to a hotel, and five of these he considered to be carcinomata. All were found in old hens, and in three thousand under one year of age no tumors were found. In this series most of the carcinomata were glandular in type, but in one the epithelium occurred in solid masses. One of the epithelial tumors was inoculated into thirteen hens and two pigeons with negative result. In one case there was a round-cell sarcoma that involved the small intestine, the mucosa of which was destroyed in places. The cells of this tumor presented a relatively small amount of cytoplasm and large spherical nuclei. In addition to the above malignant growths two fibromata were described by this author.

In one of the cases studied by Warthin[6] a leukemia was associated with tumor nodules in various organs. The normal cells of the blood were to a large extent replaced by cells of the large lymphocyte type, more or less atypical, but identical

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with those of the nodules. The latter resembled closely the tumors previously found by Butterfield[7] in hens free from leukemia. Warthin concludes, therefore, that "aleukemic and leukemic forms of lymphocytoma exist not infrequently in the common fowl, in all respects analogous to the similar condition in man." He points out that the conditions appear to be related, or are stages of the same process, and that from their nature they are to be regarded as neoplasms. The problem of leukemia then becomes identical with that of malignant neoplasms in general. More recently Ellerman and Bang[8] have injected an emulsion of the organs of leukemic hens into normal hens. By this procedure leukemia has been produced in a large proportion of those inoculated. They attempted to determine whether they were dealing with a transplantation of cells or with a condition produced by a parasite. Leukemia was produced in several normal hens by the intravascular injection of a clear and, as far as could be determined, cell-free filtrate of material derived from a leukemic hen. They, however, consider the "Infectious Leukemia of Hens" described by Moore[9] a condition distinct from true leukemia.

The first of the following tumors was noted on killing a hen, the second at autopsy some time after death, while the development of the third was followed during life.

Myxo-sarcoma.

CASE I. — Myxo-sarcoma of the thigh. The tumor was situated on the outer aspect of the thigh of an adult hen, and appeared as a turban-shaped mass with a depressed central area and an elevated rounded edge. It measured 5 centimeters across and 2 centimeters in thickness. On section it was found to consist chiefly of a translucent, soft tissue almost gelatinous in its consistence.

The tumor is found to be composed of lobules of tissue which consist of cells widely separated from one another by a relatively large amount of intercellular material. The lobules are to a large extent limited by connective tissue which in places forms a definite capsule. In certain places, however, the tumor is without capsule of any sort and appears to be infiltrating the subcutaneous fat. The central portions of many of the lobules are necrotic, and the cells in the surrounding tissue are somewhat degenerated. The connective tissue around the lobules is loose and vascular in places, and, since there is in such places a marked infiltration with leucocytes, it resembles closely granulation tissue. In it are found large numbers of lymphoid and plasma cells, and large phagocytic cells.

The cells of the tumor tissue proper are distributed throughout the matrix, which to a large extent separates them one from another, although their processes frequently anastomose. They also sometimes occur in more or less compact masses. They vary in size and shape. Most cells are elongated, some with thin tapering ends, others of uniform thickness for the greater part of their length. In cross section some are rounded, and others are flattened. The cytoplasm of many of the elongated cells is cleft at one extremity so as to form two processes extending in approximately the same direction from the nucleus. In certain portions of the tumor most of the cells are stellate, but there are also cells which are ovoid or approximately spherical. The cytoplasm of the tumor cells appears as a close reticulum, but is frequently somewhat vacuolated. In those portions of the tumor which are infiltrating the subcutaneous fat, the cytoplasm is vacuolated throughout. It seems probable that the tumor cells have taken up fat from the fat tissue which they are invading. They retain their elongated, fusiform shape, however, although they are greatly distended with globules. A smaller number of fat cells occur within the infiltrating portions of the tumor, but these are found near the surface of the lobules and probably persist only for a short time. The tumor cells are always readily distinguished from the fat cells, and there are no transitions. In those portions of the tumor which show degeneration the cytoplasm is often vacuolated. The nuclei of the degenerating tumor cells are rounded and usually oval. The linin reticulum is extremely delicate so that the nuclei appear pale and hollow. Usually there are two large masses of chromatin in each nucleus. Mitotic figures occur frequently in this tumor, and those observed were typical. Some of the tumor cells are multinucleated, but these cells occur chiefly around the necrotic portions and are evidently degenerating. Elongated cells with two nuclei occur frequently throughout the tumor. There is great variation in the size of the nuclei as well as in the size of the cells.

The intercellular material which forms the bulk of this tumor consists of a delicate fibrillar reticulum enmeshing a clear homogeneous substance which reacts to certain staining reagents like mucin. The chemical tests for mucin were not applied to the fresh tissue, but in sections stained with thionin, following Hoyer's method, the homogeneous material was stained red while the fibrillar material was stained blue. In sections stained with alum hematoxylin, the homogeneous material was stained pale blue. The fibrillar material forms a peculiar cobweb-like reticulum. The fibrils are delicate, branch, and anastomose freely, and in places tend to be rather wavy. They have less affinity for the eosin stain than do collagen fibrils, and stain bluish with alum hematoxylin. They are stained, however, a vivid blue with Mallory's connective tissue stain, so that it appears probable that they are closely related to the collagen fibrils. With the phosphotungstic acid hematoxylin stain, straight delicate fibrils are found in close relation to the tumor cells. These may possibly be fibroglia fibrils, but if so they are more feebly developed than in the connective tissue around the tumor. In the necrotic areas of the tumor the fibrillar reticulum persists longer than the cells, although in places there is evidence of softening.

The cells of this tumor present the characteristics of connective tissue cells, and are associated with intercellular material consisting in part of a fibrillar reticulum, in part of a homogeneous substance which reacts to staining reagents like mucin. The fibrillar material is probably closely related to collagen, although it is stained somewhat atypically with alum hematoxylin. The tumor is malignant in that it is rapidly growing, and in that it infiltrates normal tissues such as fat and nerves. Although blood vessels of considerable size are found within many of the lobules, the tumor tissue is not everywhere well vascularized, and is prone to necrosis. No metastases were found.

Lymphoma associated with lymphatic leukemia.

CASE II. — Lymphoma disseminated throughout the liver, the kidneys, and the peritoneum; lymphatic leukemia. This condition was found in a hen, at least one and possibly two years of age. The liver was greatly enlarged and presented many distinct rounded nodules of pale pink color distributed through its substance. Many of these nodules were apparent in the surface of the liver. Some presented slightly depressed central areas, and elongated rounded borders. The largest measured 3 centimeters across. The spleen was several times its normal size, and was rather soft. The cervical lymph nodes were enlarged. The kidneys were enlarged and studded with rounded pink nodules, some of which were 4 millimeters in diameter. Distributed over various portions of the peritoneal surface were elevated plaques of tissue from which small blood vessels radiated into the surrounding normal tissue.

The tissue of this case was poorly preserved, so that it is impossible to determine the finer histological features of the cells. It is apparent, however, that the nodules in the liver, the kidneys, and the peritoneum are composed of cells somewhat larger than the lymphoid cells of the circulating blood of the fowl, and possess a more or less spherical, eccentrically situated nucleus. They correspond quite closely in their morphology to the plasma cell, and are evidently closely related to cells of the lymphoid series. The blood found in the vessels of various organs and tissues contains large numbers or these cells, so that the proportion of white cells in the circulating blood is very great. In addition to the nodules of tumor tissue found in the liver and kidneys, these organs are to a large extent infiltrated with tumor cells.

In this fowl, therefore, cells identical with those of the tumor nodules are found in the circulation. The multiplicity of tumor nodules may readily be accounted for by the transportation of tumor cells from one part to another by way of the blood stream. More extended observations on similar cases are necessary in order to determine whether the multiplication of the cells concerned is primarily in the blood stream with secondary extension to the extravascular tissues, or is primarily extravascular with secondary extension to the circulating blood. In the case described by Warthin, no evidence of cell division was found in the blood, although mitoses were found in the nodules.

Lymphoma without lymphatic leukemia.

CASE III. — Lymphoma of the neck with metastasis to the lungs and the liver. This tumor was noted as a small swelling on the neck of a hen about one year old. (For this case we are indebted to Mr. Moses Hemingway, Emery Mills, Me.) It increased gradually in size up to the time it came under personal observation six months later. At that time it appeared as an oval flattened mass with an elevated rounded edge and a thick central crust. It measured 5 x 6 centimeters across and 2 centimeters in thickness. A thin slice of the tumor was excised for diagnosis, and it was found to be composed of rounded cells, which varied considerably as to size, with rounded, somewhat irregular nuclei. Nine days later the tumor was excised, portions of it were placed in various fixatives, small bits were inoculated into the subcutaneous tissues and muscles of a cock and two hens of the same breed and flock and of about the same age, and another bit was inoculated into the pectoral muscle of the hen in which the tumor originated. The tumor apart from necrotic areas was of uniform pale pink color, and appeared homogeneous throughout.

The hen was light in weight and showed weakness and labored breathing before the operation. Subsequent to this there was improvement, but this was of short duration. Seven weeks after the operation there was great emaciation, cough, and diarrhea, so that she was chloroformed and autopsied at once.

There is recurrence at the site of the operation, where the tumor appears to be growing diffusely through the tissues about the scar. The pectoral muscle at the site of inoculation is an indefinite nodular mass differing in color from the normal muscle. The liver is greatly enlarged. Its right lobe presents a rounded nodule, 1 centimeter in diameter, consisting of tissue of similar appearance to that of the primary tumor. The entire liver is infiltrated with this tissue which appears to augment the size of the lobules and in places grows diffusely in areas 2 to 5 millimeters in diameter. The tumor involves both lungs, and only a small portion of one lung contains air. Near the roots of the lungs are two rounded nodules of tumor tissue. The other organs appear to be not involved.

The tumor consists of cells with little supporting tissue. The latter consists for the most part of a delicate reticulum of connective tissue which apparently does not develop to any considerable extent within the tumor, but represents preëxisting tissue into which the tumor has grown. There are also a few coarser bands of fibrous tissue. The tumor is rather vascular throughout, and the connective tissue reticulum is more distinct around the vessels than elsewhere. The tumor cells vary in size. Many are no larger than the lymphocytes of the circulation, but some are equal in size to large connective tissue cells. They are apparently not concerned in the formation of intercellular fibrils. The cell boundaries are ill defined, but the majority of the cells are either rounded or irregularly polyhedral from mutual compression. The nuclei are likewise spheroidal or ovoid in form. Mitotic figures are everywhere numerous. Throughout the primary tumor are cells of about the same size as the larger tumor cells, but with a cytoplasm which stains more intensely with Unna's alkaline methylene blue. These cells are often irregular in outline as though fixed when in ameboid motion, and are found protruding through the walls of blood vessels. They are not found in the metastases, and are therefore interpreted as cells which have migrated from the circulation into the primary tumor probably on account of the areas of necrosis present. In certain portions there are large numbers of eosinophiles. The endothelium of the blood vessels in the primary tumor is indefinite in many places, while in others it appears to be entirely wanting so that the blood appears to lie in spaces within the substance of the tumor. Wherever the endothelium is apparent the tumor cells are found in actual contact with it, a relation which favors dissemination of tumor cells by the blood stream.

The tumor has infiltrated the greater portion of the lung. In the least affected parts it is distributed in a thin layer between the lobules, and also about the centrally situated terminal bronchus. In both these situations it is perivascular, and the tumor cells are found growing through the loose connective tissue around the larger vessels. As the tumor continues to encroach on the normal structure of the lobule its cells are found growing between the capillaries throughout the walls of the air sacs. After the air spaces are entirely obliterated the capillary network persists for a time and serves to vascularize the tumor. With the continued growth of the latter the capillaries are gradually obliterated and no trace of the structure of the lung lobule remains. The tumor often infiltrates the smooth muscle and the epithelium of the terminal bronchi. In those where the normal tissues are being invaded by the tumor there are large numbers of degenerating and disintegrating cells, which appear to belong to the tissue of the lung.

The tumor has extended into all the large blood vessels of the lung where it has grown in a continuous layer beneath the endothelium. Many tumor cells are found free in the blood of the vessels, but they apparently find the most favorable conditions for their growth in close relation to the endothelium. The cells in this situation are of larger and more uniform size, and show little evidence of degeneration. In poorly vascularized portions of the tumor the cells are small and the nuclei are pyknotic.

The great enlargement of the liver is the result of infiltration of the entire organ with the tumor tissue. The greatest development of the tumor tissue is parietal with relation to the liver lobules, so that it is probable that the tumor cells were brought to this organ through the hepatic artery. The tumor appears in rounded confluent masses which contain the portal vessels and bile ducts. With the growth of the tumor tissue the liver lobules become compressed and irregular or flattened. In places there is a network of liver tissue, the spaces between the anastomosing lobules being filled with tumor. The surface of the tumor masses is invariably convex, while that of the liver tissue is concave. In the portions most involved flattened or stellate islands of the liver parenchyma are the only remnants of the normal structure. The tumor cells are also diffusely distributed throughout the liver tissue that remains, in which they are situated beneath the endothelium of the sinusoids as well as in the spaces of the sinusoids. The tumor is found growing beneath the endothelium of the large vessels, and large numbers of tumor cells occur free in the blood of many large vessels. These cells apparently did not occur throughout the general circulation for they were not found in the vessels of organs other than the lung and the liver.

The portions of this tumor which were inoculated into a cock and two hens became absorbed after a short time. All were kept under observation for several months, but developed no tumors. A bit of the tumor inoculated into the pectoral muscle of the hen in which it originated developed to the size of a small pea during the seven weeks following its implantation. This repeats in another species the observation made by Loeb[10] in the transplantation of the mixed tumors of the mamma of dogs. The tissues of the animal in which the tumor originated furnishes a more favorable medium for growth than do the tissues of normal individuals of the same species.

In comparing this tumor with that of the preceding case, we find an essential difference in that in one the tumor cells are capable of existing disassociated and free in the circulating blood as well as in connected masses in the tissues, while in the other there is ample evidence to show that large numbers of tumor cells enter the blood stream from the fixed masses of tumor tissue but do not continue in the general circulation so as to produce a condition of leukemia.

For the material and histories of the following six cases we are indebted to Dr. Leary, Professor of Pathology, Tufts Medical School. None of the four cases in which there were lymphoma nodules presented a leukemia.

CASE IV. — Lymphoma of the neck infiltrating the sternum and the adjacent muscles with metastases to the lung and the liver.

The tumor developed in a white leghorn chicken, somewhat less than a year old, which was kept at the laboratory for the purpose of inoculation. The chicken was noted to be in rather poor condition, and was frequently found pecking at the side of the neck where the external tumor was discovered.

The tumor situated on the neck consists of a rounded nodule measuring 3.5 x 3 x 2.5 centimeters. It is elevated about 1.5 centimeters above the surrounding surface and is covered with skin except for a crater-like depression in its outer surface. The tumor is of firm consistence and the skin covering it is without feather follicles, except at one side. The skin is tense and adherent to the nodule. On section the tumor appears grayish pink, somewhat translucent. and homogeneous. At the margin of the crater-like depression there are dark brown or blackish crusts. About 2 centimeters from this tumor and in the region of the sternum, there is a smaller mass which is firmly adherent to the bone and adjacent muscle.

The liver weighs 49 grams (after preservation by the Kaiserling method). On the dorsal surface near the inferior border there is a sharply circumscribed, grayish white, umbilicated mass measuring 2.8 x 2.5 x 2 centimeters. This is elevated slightly above the surrounding surface of the liver which is light brown in color. On the ventral surface of the liver this mass shows through as an irregular, ill-defined area measuring about 1 centimeter in its greatest diameter. On section the tumor tissue appears grayish pink in color, homogeneous and of firm consistence. It is sharply limited from the surrounding liver tissue. The other organs appear normal.

On microscopical examination the tumor of the neck is found to consist of masses of moderate sized lymphoid cells vaguely divided into large lobules by a few septa of connective tissue. Through it extend moderate sized blood vessels and numerous capillaries around which the tumor cells are better preserved than elsewhere. Many of the cells resemble those of the germinal centers of lymphoid follicles. Others resemble plasma cells and possess rounded or oval vesicular nuclei. Scattered here and there are cells with large, pale, oval nuclei resembling those of endothelial cells. The tumor cells in general have a small amount of homogeneous cytoplasm which stains pink with eosin. Mitotic figures are numerous. There is a small number of polynuclear leucocytes scattered throughout the tumor tissue. The blood appears normal. At the periphery of the tumor and separated from it by a definite though thin capsule of connective tissue, there are a few muscle fibers which have apparently been displaced by the growth of the tumor mass.

The presternal mass consists of tumor tissue similar to that of the nodule of the neck. The tumor has infiltrated the muscle and has penetrated the bone, the marrow of which is practically replaced by tumor cells.

The lung presents large rounded masses of tumor tissue similar to that already described. In certain sections there is merely a small remnant of lung tissue about the tumor nodules. There are numerous mitotic figures in some places, but in other areas the tumor cells are irregular and apparently degenerating.

The nodule in the liver consists of lymphoid tissue of the same character as that of the primary growth. The liver tissue at the periphery of this nodule is infiltrated with tumor cells and presents various degrees of degeneration.

In this case there is a primary tumor of the nature of a lymphoma, situated on the neck, with secondary nodules on the sternum, in the lung, and in the liver. The sternum and adjacent muscles are infiltrated with the tumor, but the tumor occurs as discrete nodules in the lung and liver. The spleen is not involved and the blood appears normal.

CASE V. — Lymphoma involving the small intestine, spleen, lung, liver, and kidney.

This hen was from a flock in which there had been several cases of amebiasis. An examination of the blood showed a considerable increase in the number of leucocytes, which were chiefly eosinophiles. The gross appearances were not noted.

In sections of the lung there are small masses of lymphoid cells which in places appear to distend the peribronchial lymphatics. On the pleural surface is a large nodule of lymphoid tissue at the periphery of which are smaller nodules of similar character. They resemble somewhat the active follicles of lymph nodes. In places there are large numbers of eosinophiles associated with the masses of lymphoid cells.

The liver presents a varied picture. Scattered in the portal spaces are rounded masses of lymphoid cells among which there is a delicate reticulum. These masses are sharply circumscribed and appear to distend the perivascular lymphatics. In other places, however, there are groups of lymphoid cells trailing off between the liver cells. These are for the most part associated with large numbers of eosinophiles. Many such areas have necrotic centers, the cells at the periphery only being recognizable. The necrosis is diffuse and somewhat closely resembles caseation. In other parts of the liver, the central portion, similar foci consist of connective tissue with an occasional giant cell. Sections were stained for tubercle bacilli but none were found. No fresh material was available for the purpose of inoculation.

Throughout the spleen are rounded nodules of lymphoid cells, similar to those found in the lung and liver. They are apparently situated in the lymphatics and are easily distinguished from the splenic follicles.

Sections of the small intestine present several small rounded nodules of lymphoid tissue situated in the mucosa and submucosa. They are sharply circumscribed and differ from the normal lymphoid follicles. There are also collections of lymphoid cells scattered throughout the kidney. The blood appears normal.

In this fowl, sharply circumscribed masses of lymphoid cells are found in the lung, liver, spleen, intestine, and kidney. They often appear to be situated in the lymphatic vessels which they distend. In the lung these masses resemble somewhat the normal lymphoid follicles, but in the spleen and intestine they are readily distinguished from the latter. In them, endothelial cells are occasionally found and some of these are phagocytic. In the liver are large and small necrotic or scirrhous areas about the periphery of which are large numbers of lymphoid cells and eosinophiles. Whether there are two distinct processes, one inflammatory and the other neoplastic, or whether we are dealing with a lymphoma, which, in the liver, is of scirrhous type, we are unable to decide. The process may be a chronic inflammatory one, similar to tuberculosis or syphilis in the human being, but it resembles a tumor in many respects. Unfortunately fresh material was not available for smears, cultures, and animal inoculations.

CASE VI. — Lymphoma. The gross appearances were not noted. The tumor consists of cells indistinguishable from those of the lymphoid series. Many of the cells resemble plasma cells, and there are in places small numbers of eosinophiles, especially in the vicinity of the blood vessels. The tumor is diffusely infiltrating the fat tissue. Here and there, there is a suggestion of a reticular tissue between the tumor cells, and there are a few coarse strands of connective tissue in the vicinity of the blood vessels.

No tumor tissue is found in sections of the liver and spleen.

The situation of this tumor could not be ascertained, but in sections it is found infiltrating adipose tissue. It is of the nature of a lymphoma, and contains numerous eosinophile cells. There is no evidence of metastases.

CASE VII. — Lymphoma growing diffusely in the liver, lung, mesentery, and intestine. A Rhode Island red hen about eight months old had been observed to be emaciated and sickly for some time. No external tumors were found. This hen was of the same flock as Case VIII. At autopsy, large, light grayish nodules were found in the liver, lungs, mesentery, and intestine.

The liver weighs 108 grams (after preservation by the Kaiserling method). It is somewhat enlarged. The lobes are approximately of equal size, the left measuring 10.5 centimeters in length and 4.5 centimeters in width, and the right 9.5 centimeters in length and 4 centimeters in width. The greatest thickness is 3 centimeters. The ventral surface of the lower two-thirds of each lobe consists of a mass of tissue of grayish yellow color, with a fine capillary injection, and an irregular margin. This is nodular and sharply demarcated from and elevated above the more normal liver tissue, which is reddish brown in color. Scattered throughout the liver are numerous nodules 2 to 5 millimeters in diameter with rounded surface, projecting slightly from the surrounding tissue. The color, consistence, and general appearance of these scattered nodules are similar to the marginal mass above described. On the posterior surface the general arrangement of the tumor is similar. On section the nodules are sharply limited from the surrounding liver tissue.

On histological examination the liver is found to be poorly preserved. Hepatic cylinders are difficult to recognize, and are, to a large extent, replaced by evenly distributed cells of the lymphoid type, among which are areas of necrosis.

The tumor in the mesentery is composed of cells which in general resemble those of the tumor in the liver, although they show greater variation in size. In places where the cells are small and closely packed together there is a suggestion of alveolar arrangement. There are areas of necrosis with numerous polynuclear leucocytes.

In the serosa of the small intestine there are discrete, rounded masses and diffuse areas of lymphoid tissue. The muscle layer is infiltrated throughout, and in certain places the tumor reaches the mucosa.

The normal structure of the lung is entirely obscured by diffuse lymphoid tissue which occasionally shows slight evidence of alveolar arrangement.

The lung, liver, mesentery, and small intestine in this hen were infiltrated with a diffusely growing lymphoma which presents here and there a suggestion of alveolar arrangement.

CASE VIII. — Lymphoma of the liver and kidneys. A young adult hen of large size from the same flock as Case VII. was brought while alive to the Pathological Laboratory of Tufts Medical School. It was in poor condition though not much emaciated.

The liver was found by Dr. Leary to be enlarged and distinctly palpable. The hen was anesthetized, a small piece of the liver was removed, and inoculated subcutaneously and intraperitoneally into a sister hen. No visible growth resulted, and, when this bird was killed some months after the inoculation, no trace of the tumor tissue was found. The first hen died shortly after the operation. At autopsy the liver and the kidneys were found to be of great size.

The liver weighs 376 grams (after preservation by the Kaiserling method). Its transverse diameter is 10 centimeters, length of right lobe 14.5 centimeters, left lobe 13 centimeters, and its greatest thickness is 5.5 centimeters. The ventral surface is of a general grayish brown color with irregular, lighter, grayish yellow mottling, with a delicate injection of the blood vessels. The surface is smooth. Near the tip and median edge of the left lobe are two irregular, bright yellow areas .6 x .3 centimeter in diameter. These are slightly elevated and sharply demarcated from the surrounding surface. On section the liver tissue at the periphery is of a bright reddish color. Posterior surface of the liver is similar to the anterior. In the posterior median portion of the right lobe is a ragged, blood-stained cavity 2 x 2.5 centimeters in diameter where a mass of tissue had been removed during life for the purpose of inoculation and histological examination.

The kidneys weigh 65 grams (after preservation by the Kaiserling method). They are 8.3 centimeters long, 3 centimeters wide, and 3.5 centimeters thick. The tri-partate structure is clearly shown. In general the surface is smooth anteriorly, and lobulated posteriorly to the contour of the bony frame work. On section the kidneys are grayish pink in color, translucent and fairly firm in consistence. On section the surface shows a few large injected blood vessels scattered generally throughout the organ, which become smaller at the periphery. The spleen is not enlarged and appears normal.

Histology. — The hepatic cylinders are almost entirely replaced by small and large nests of lymphoid cells. These cells somewhat resemble those in the germinal centers of lymphoid follicles. Most of the liver cells which can be recognized are swollen and vacuolated, or necrotic.

There are mitotic figures in many of the tumor cells. Many of the alveoli of tumor cells are surrounded by a layer of flattened endothelial cells. The centers of some of such masses are necrotic. Others appear empty containing only trabeculæ similar to a reticulum. All transitions between these conditions occur. Although the general arrangement of the tumor is alveolar there are tumor cells scattered diffusely between the remaining hepatic cylinders. In certain places there are large, sharply circumscribed, necrotic areas (infarcts), about which there is a small amount of connective tissue, intermingled with regenerating bile ducts and liver cells. The alveolar arrangement is marked and somewhat suggests carcinoma. The tumor cells are particularly well preserved in the vicinity of these infarcts.

The kidneys are diffusely infiltrated with lymphoid cells. In places only a few necrotic tubules are distinguishable. The tissue is very poorly preserved so that histological details cannot be determined.

The blood appears normal.

The great weight of the liver and the kidneys in this case are due to the great amount of tumor tissue with which they are infiltrated. In the liver the lymphoma presents an alveolar arrangement, although there is also a diffuse distribution of the tumor cells, whereas in the kidneys it is everywhere diffuse in its growth.

Leiomyoma.

CASE IX. — Leiomyoma of the mesentery. This tumor was found accidentally in a hen purchased in a market. Specimen consists of a loop of hypertrophied intestine 30 centimeters long and 3 to 4 centimeters in circumference. Within the mesentery and covered on both sides by peritoneum is a large, rounded tumor of firm, flesh-like consistence, which measures 7 x 5 x 5 centimeters. This is of a general reddish pink color. On section the coloring is similar, and the surface is traversed by firm, glistening bands of somewhat lighter color. On scraping the surface these stand out prominently and no soft tissue comes away. Between this tumor and the intestine is a tumor mass of similar character measuring 2 x 1.5 centimeters.

On histological examination the tumor is found to be composed of typical smooth muscle fibers running in various directions. Sections stained by the Van Gieson method for connective tissue show only a few small strands of fibrous tissue between the masses of smooth muscle fibers.

This tumor is composed chiefly of smooth muscle fibers, and is in many respects similar to the leiomyoma of mammals.

On account of the common practice of killing fowls which have been kept for laying at the age of one or two years, it is probable that comparatively few attain great age. Two of the hens in the present series were known to be less than a year old. Considering, therefore, the age ordinarily attained by common fowls it would appear that tumors are of frequent occurrence with them. Cases VII. and VIII. were both from the same flock, and were probably closely related. In the flock from which Case II. was taken another hen which died soon after also presented tumor-like nodules in the liver and kidneys. On microscopical examination, however, these were found to consist of inflammatory tissue, and were evidently not new growths. There was no other recognizable disease in either of these flocks.

It is probable that with the continuation of the investigation of tumors in the lower animals additional types of tumors will be found in each species. Three types of carcinoma have already been described in the common fowl — squamous-cell, alveolar, and glandular cancer — and also benign tumors of the nature of fibromata. In the present series two other varieties of new growth have been described, — myxo-sarcoma and leiomyoma.

In the total number of tumors thus far studied in fowls, lymphoma, however, appears to occur most frequently. It is possible that this tumor develops in younger birds than certain other tumors, and this may account for its relative frequency. In some cases lymphoma occurs as a local primary growth either with or without metastases. In other cases the tumor tissue is so uniformly disseminated throughout certain organs that it is impossible to determine the point of origin. Certain lymphomata are more or less alveolar in structure, others grow diffusely through the tissues. The tumor may be confined to the fixed tissues so that it is essentially extravascular, or the tumor cells may also occur in the circulating blood constituting a lymphatic leukemia. In the case published by Warthin no mitotic figures were found in the tumor cells in the blood, and in Case II. of the present series the preservation was so poor that none could be distinguished.

These data, together with the previous observations of Warthin, suggest certain theoretical considerations concerning the relation of leukemia and tumor growth. They tend to amplify the concept already held that leukemias are tumors which have invaded the circulating blood. It is well known that the cells of epithelial tumors may enter the blood stream and be transported to various parts of the body. It is impossible, however, for such cells to exist free in the circulation. Ordinarily their size alone prevents them from passing freely through the capillaries. Furthermore, epithelial cells tend to grow in continuous masses and to become attached to the supporting tissue. In the histological study of a series of implants of a mouse tumor the epithelium, living when inoculated, shows a progressive necrosis of the central portion of the implanted mass up to the time it comes into actual contact with the connective tissue of the host. Likewise the cells of tumors which produce intercellular substance obviously cannot multiply indefinitely in the blood stream, although it is well established that the cells of such tumors frequently enter the circulation and in this way produce metastases.

In leukemia, on the other hand, we find an unlimited production of cells which conform more or less to types of cells found normally in the circulation or in the hematopoietic organs. Cells of these types do not produce intercellular structures, and where they occur in masses, as in the lymph nodes, in the bone marrow, and in tumors, they are supported by connective tissue. It is readily seen that with tissue of this sort either circulating or fixed tumors may occur, or both may co-exist in the same individual. The only distinction, therefore, between aleukemic lymphoma and lymphoma with lymphatic leukemia is that in the former the tumor is extravascular while in the latter the tumor is intravascular. In certain cases the tumor is both extravascular and intravascular in its growth. That many of the tumors of this type are not associated with leukemia — *i.e.*, are not intravascular — is probably due to the biological character of their cells, which is such that they are not attracted into the blood vessels or do not find it a suitable medium for multiplication. Lymphomata are most frequently composed of cells resembling more closely the cells of the germinative centers than the mature lymphocytes of the circulation. In order that they multiply in the circulating blood either the cells in question must be adapted for this medium or the blood must be modified in some way so as to furnish a medium suitable for the growth of the tumor cells.

In the case of the transplantable tumors of mice the initiative to growth resides in the tumor cells, for they grow when transplanted to normal animals. Other tumors, such as that of Case III. of those described, grow if inoculated into another part of the individual in which they originated, but fail to grow when inoculated into other individuals of the same breed. The tumor in Case VIII. also failed to grow on inoculation into other hens. Such tumors apparently demand for their growth peculiar conditions which are only found in the individual in which they arise. Whether these conditions amount only to an individual peculiarity, or whether they are abnormal, and possibly concerned in the initial growth of the tumor cannot be readily answered.

Although certain of the tumors of various species present characteristics peculiar to each, these tumors of the lower animals are analogous to those of the human being. Many of them present the same properties of malignancy — unlimited growth, infiltration of normal tissue, and metastasis. Others are of benign character and apart from specific peculiarities resemble the benign tumors of human beings. The counterpart of the lymphoma and the occasional associated condition of lymphatic leukemia in human beings is found in a similar tumor which is of frequent occurrence in the common fowl.

SUMMARY.

Tumors are apparently of frequent occurrence in the common fowl. Apart from certain specific peculiarities these are in every way analogous to the tumors of human beings.

In addition to the tumors hitherto found in the hen, myxosarcoma and leiomyoma are described in the present series of cases.

Lymphoma occurs both with and without lymphatic leukemia. The latter condition consists of the presence in the circulating blood of tumor cells having the characteristics of the cells of the lymphoid series. The tumor may occur as a definite primary growth either with or without secondary nodules, or it may be so disseminated that the site of its origin cannot be determined. Some lymphomata have a more or less alveolar structure, while others grow diffusely by infiltrating normal structures.

The inoculation of the lymphoma tissue from two of the cases into normal hens resulted negatively, but the inoculation of a bit of one of these tumors into the pectoral muscle of the hen in which it arose resulted in the development of a mass of tumor tissue at the site of the inoculation.

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DESCRIPTION OF PLATES.

Plate XLV.

Fig. 1. — Case IX. Leiomyoma of the mesentery of a hen. Two-thirds natural size.

Fig. 2. — Case IV. Lymphoma of the neck with a sharply circumscribed secondary nodule in the liver.

Fig. 3. — Case VII. Liver to a large extent infiltrated with lymphoma.

Plate XLVI.

Fig. 4. — Case VIII. Liver uniformly infiltrated with lymphoma. A thin slice excised from an infarcted area.

Fig. 5. — Case VIII. Kidneys greatly enlarged through infiltration with lymphoma.

(Figs. 2, 3, 4, and 5 about four-fifths natural size.)

Fig. 6. — Case VIII. Lymphoma infiltrating the liver, showing an alveolar structure.

PLATE XLVII.

FIG. 7. — Case I. Myxo-sarcoma. A small nodule of the tumor situated upon the surface of the larger mass.

FIG. 8. — A higher magnification of the same tumor. The upper portion is taken from a section stained with Mallory's iron hematoxylin, and shows the cells and not the fibrils. The lower portion is taken from a section stained with alum hematoxylin, and shows the character and amount of fibrillar material in the tumor.

FIG. 9. — Case III. Lymphoma infiltrating the liver. Rounded masses of tumor tissue occur, between which are the compressed strands and layers of liver tissue.

FIG. 10. — Case III. Lymphoma infiltrating the wall of a terminal bronchus. The tumor cells have replaced the bronchial epithelium.

FIG. 11. — Case III. Lymphoma infiltrating the pectoral muscle. This mass of tumor tissue developed from a small bit of the primary tumor, which was transplanted to the pectoral muscle of the same hen.

PLATE XLVIII.

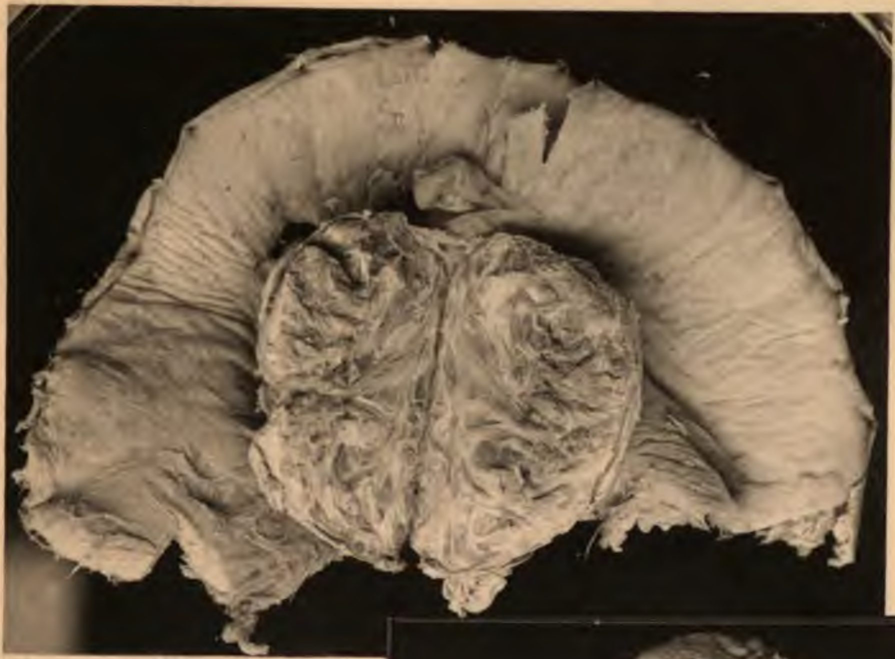
FIG. 12. — Case II. Lymphoma. This nodule was taken from the liver.

FIG. 13. — Case II. A small mass of lymphoma tissue in the kidney. The glomerulus in the lower portion of the figure is also infiltrated. In this case the tumor cells occurred in large numbers in the blood, constituting a lymphatic leukemia.

FIG. 14. — Case V. A nodule of lymphoid tissue, evidently a lymphoma, in the surface of the lung.

FIG. 15. — Case IV. Lymphoma of the liver. A sharply circumscribed nodule.

FIG. 16. — Case VII. Lymphoma infiltrating the muscle layers of the small intestine.



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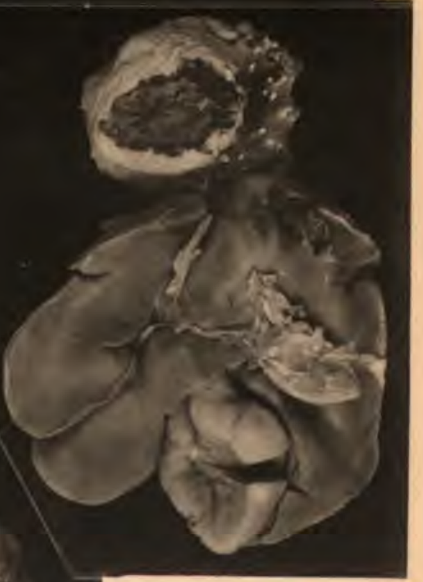

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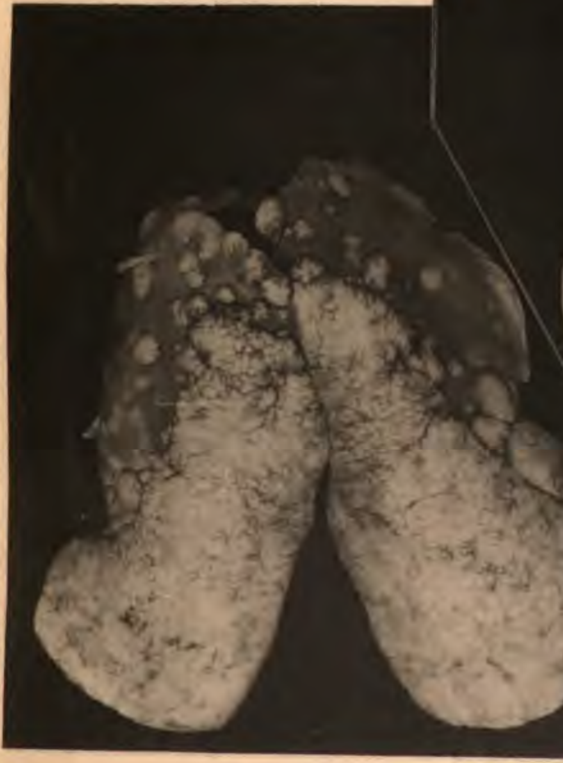

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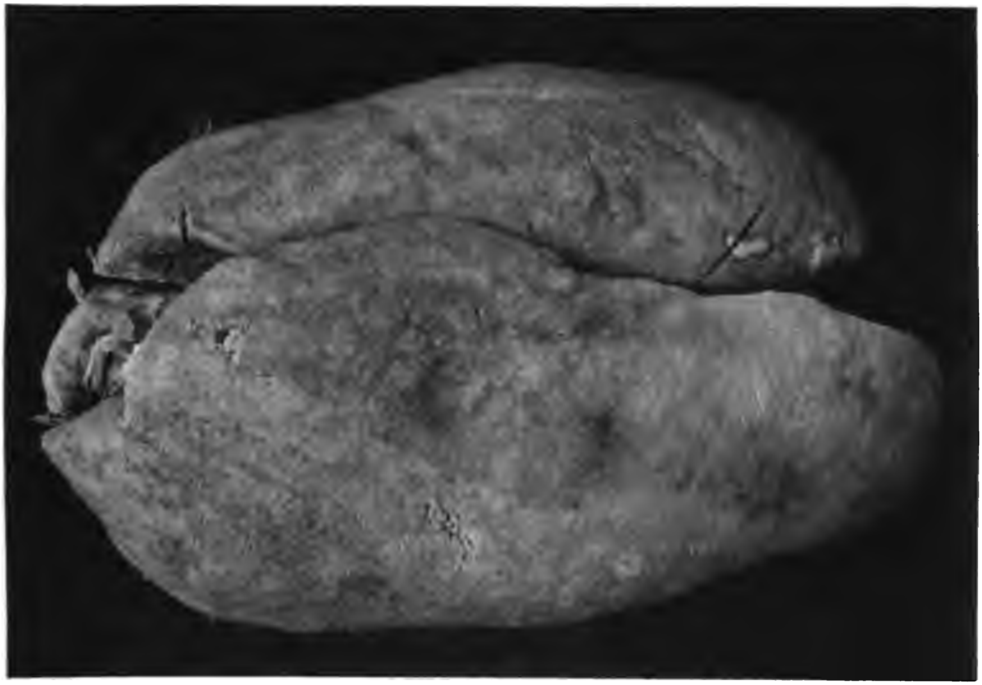

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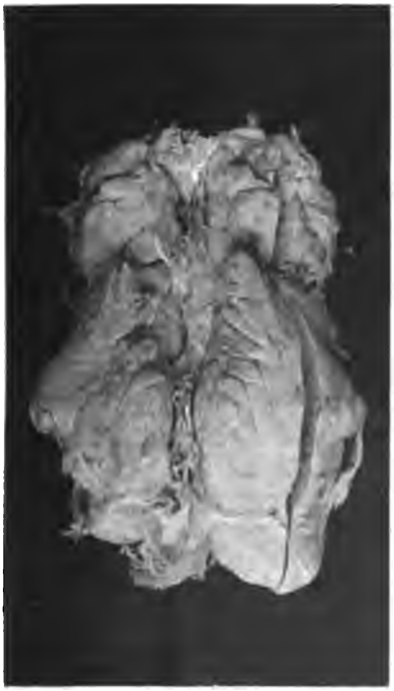

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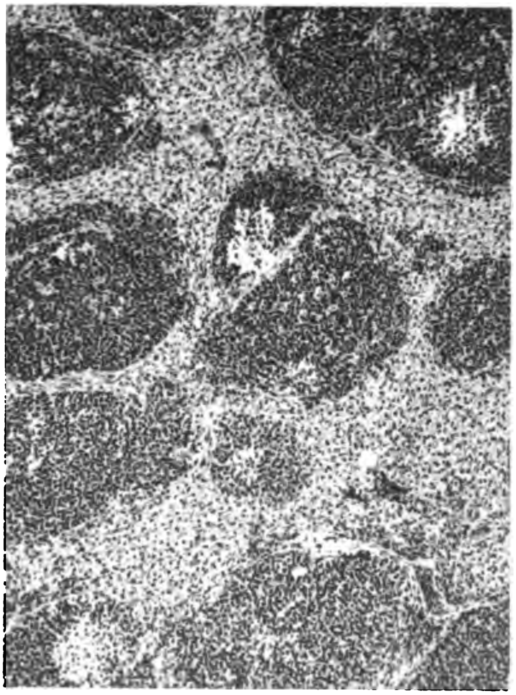

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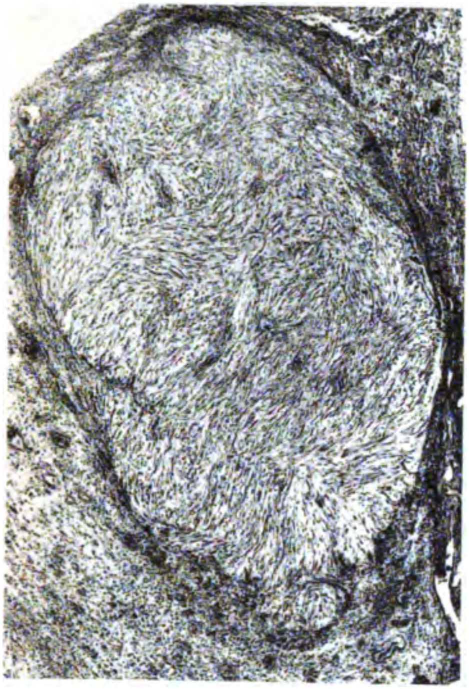

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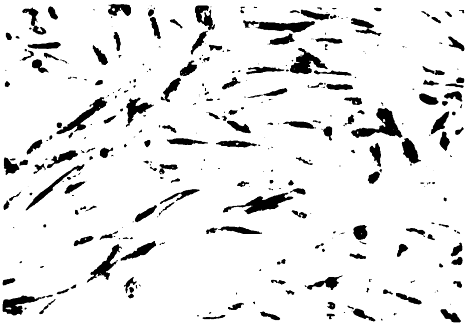

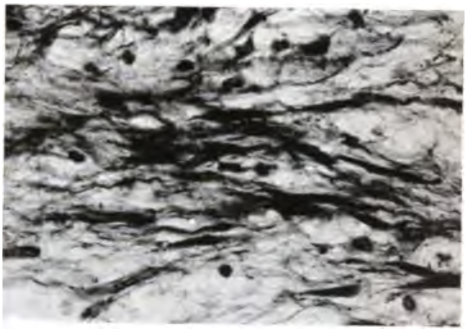

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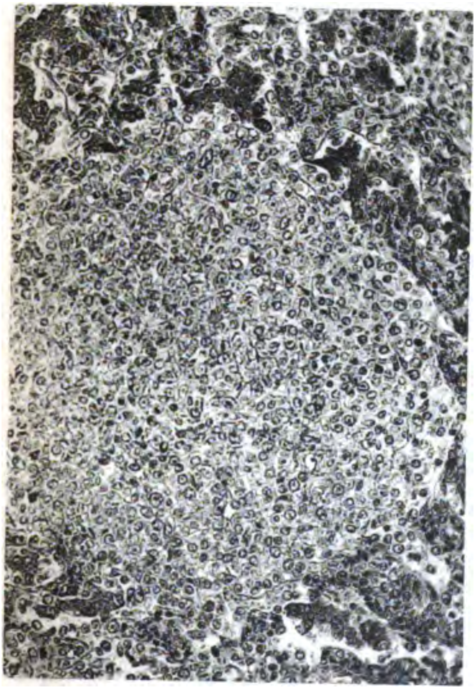

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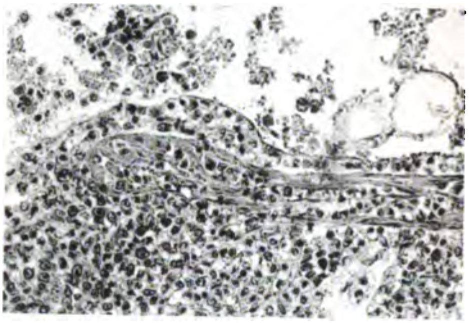

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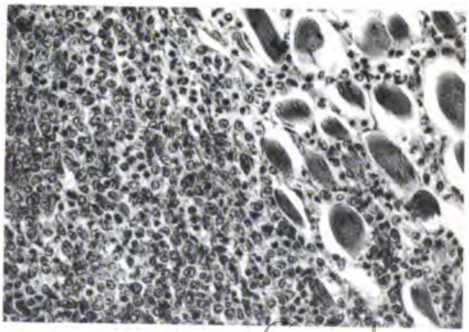

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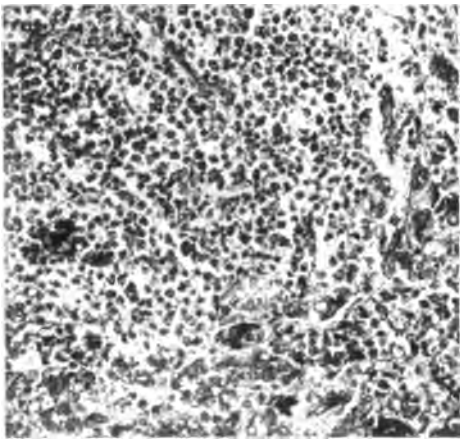

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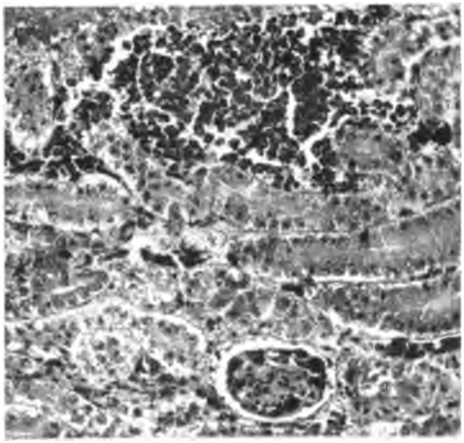

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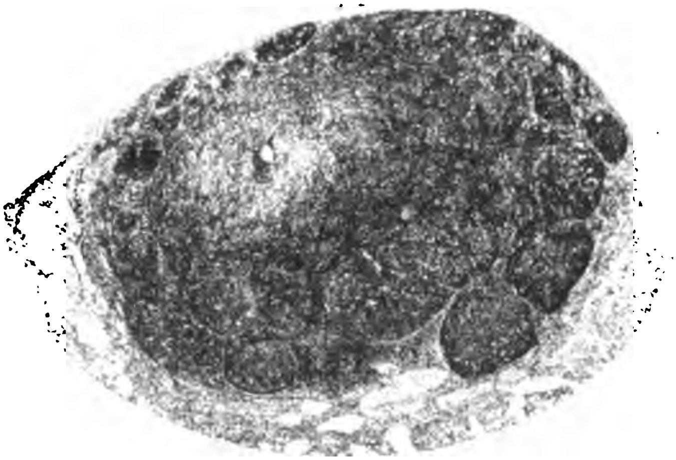

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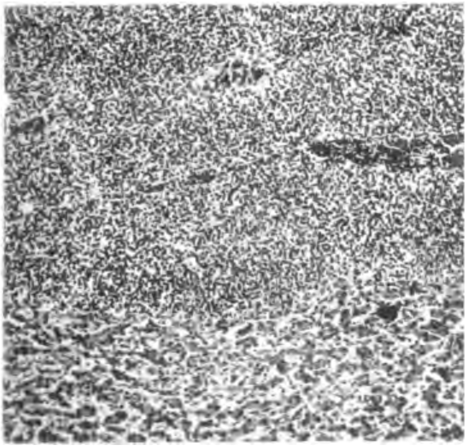

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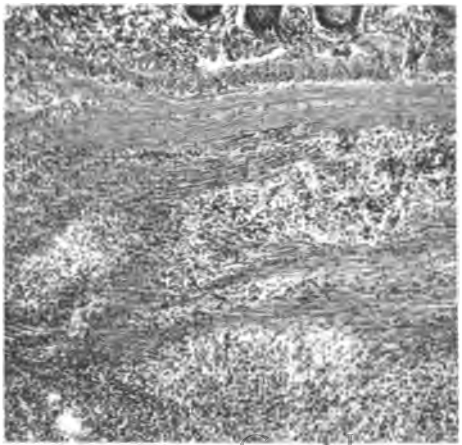

16

A SERIES OF SPONTANEOUS TUMORS IN MICE WITH OBSERVATIONS ON THE INFLUENCE OF HEREDITY ON THE FREQUENCY OF THEIR OCCURRENCE.*

E. E. TYZZER.

In a previous communication † attention was called to the frequency with which tumors were found in certain families of mice. The occurrence of other tumors in these same families and in other mice which have been kept under observation has furnished additional data. This series of spontaneous tumors will serve also to show the frequency of internally situated tumors in mice, most of which are unsuspected during life and all of which pass unobserved in case no autopsy is performed. It was only through the practice of making a more or less complete post-mortem examination of each mouse which died in the laboratory, whether killed intentionally or dying from natural causes, that the greater proportion of the tumors were found. Moreover, all nodules or lesions of doubtful nature were sectioned and examined histologically. Since in this series there are so few tumors of mammary origin, and since these have already been described and classified, only brief reference will be made to them. Certain types of tumors hitherto unrecorded, as well as others which from their character and frequency occupy an important position among the tumors of mice, will be described in full.

Including the series of twenty previously reported, a study has been made of eighty-three different tumors which occurred in seventy mice. Ten of these mice had each two primary tumors of different types, and one had primary tumors of four different types, namely, — papillary cystadenoma of the lung, hypernephroma, lymphoma, and adenocarcinoma of the ovary. Thus over fifteen per cent of

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† Fourth Report of the Cancer Commission of Harvard University, 1907, 71. Jour. Med. Research, 1907, XVII., 199.

the mice of this series possessed two or more primary tumors which differed in type from one another. In addition to these, it is possible that the primary tumors of the lung were multiple in several cases, and there were double primary ovarian tumors in two cases, but these tumors were of the same type.

Of this series of eighty-three tumors, eleven were external; of these eleven, eight were epithelial tumors, — probably mammary in origin; two were of the nature of lymphoma; and there was one sarcoma of the orbit. The statement of Apolant[1] that ninety-five per cent of all tumors in mice are tumors of mammary origin is not borne out by this series, in which less than ten per cent of the tumors are of this type. Primary tumor of the lung was present in fifty-two cases, lymphoma in ten, primary tumor of the kidney (hypernephroma) in four, papillary cystadenoma of the ovary in four cases, sarcoma of the liver in one, and sarcoma of the orbit in one case. The primary tumors of the lung, therefore, constitute sixty-two per cent of the tumors of this series, and, since they were associated with other primary tumors in certain cases, they were found in somewhat over seventy-four per cent of the seventy mice in which tumors occurred.

Most of the tumors arising in the lung of the mouse are of the nature of papillary cystadenoma. Livingood[2] was the first to describe this tumor in a mouse, and its occurrence in mice with inoculated tumors was subsequently noted by Haaland.[3] I[4] subsequently found this tumor occurring with about equal frequency in inoculated and uninoculated mice, and called attention to the fact that spontaneous tumors may develop in animals which have been found refractory to inoculable tumors. Primary tumors of the lung were found by Jobling[5] in three mice which also possessed adenocarcinoma of the mammary gland.

Primary tumors of the lung vary in size from minute nodules barely visible to the naked eye to tumors occupying one-third or more of the space of the thorax. Several very minute tumors of this type were not observed at the autopsy, but were discovered accidentally in stained sections. They are usually situated in the surface of the lung beneath the pleura, but several have been found in the interior of the organ. There is nothing in their gross appearance by which they may be distinguished either from the metastases of tumors, primary elsewhere in the body, or from the nodules of inflammatory tissue which have been frequently noted in the lungs of the mice kept under observation. In several instances primary tumors have been unexpectedly found associated with recognized infectious processes from which they could not be distinguished without histological examination. The primary tumors of the lung show considerable structural variation, and this may be true for different portions of a single tumor as well as for different tumors. Notwithstanding individual variation, most of them present certain structural features so that they may be classed as papillary cystadenomata. However, certain of the primary lung tumors, on account of the irregularity of the growth of their epithelium, must be considered carcinomata. These two types of tumors will, therefore, be described separately.

Papillary cystadenoma of the lung. — These tumors consist of epithelium arranged in a single layer upon irregular folds and processes of supporting tissue. They may be compact and almost solid in their structure, or open and cystic. The space between the folds is usually filled with fluid, although there is evidence that air has free access to the interior of certain tumors, and bronchi are found opening into them (Figs. 5 and 6). Phagocytic cells occur often in the spaces between the folds and septa. The structure of the tumor thus corresponds to a certain extent to the structure of the lung. The tumor nodules may be sharply limited, while the alveoli of the surrounding lung tissue are flattened and compressed through the expansive growth of the tumor. In other instances the growth of the tumor is infiltrative in character, and its epithelium is found extending into the surrounding alveoli which become lined with tumor epithelium. The supporting tissue of the lung with its blood supply is thereby incorporated into the growing tumor, and persists to such an extent that it is often recognizable in the peripheral portion of the tumor. The exact boundaries of tumors which grow in this manner are not readily distinguished.

The tumor frequently grows into bronchi, where it usually presents a papillary structure, and this makes possible the dissemination of tumor cells throughout the lung. In several instances, in which irregular papillary processes had extended from the tumor into a bronchus, isolated cells and groups of cells were found free within the latter, and numerous small secondary tumor nodules were situated in the portion of the lung into which the bronchus led (Fig. 6). In one case the tumor epithelium was found in a peribronchial lymphatic and also beneath the endothelium of a vein. In no case, however, have unquestionable metastases of this tumor been found in other organs than the lung.

The tumor epithelium is either columnar, or cuboidal, or flattened, and is without cilia. The epithelium in places may be vacuolated, or its cytoplasm may contain minute hyaline globules. In most cases it resembles the bronchial epithelium, but it sometimes resembles the thickened alveolar epithelium such as is found in atalectatic areas.

It is often difficult to determine whether we have to deal with atalectases or true tumors. Only those cases, however, which present distinct newly-formed structures are included in this series. In a previous description the infrequency of indirect cell division was emphasized, mitoses having been found in only one of twelve cases. Mitotic figures are present in several of the forty tumors which have since been studied, and there are other evidences of growth. Amitotic nuclear division is found to a greater or less extent in practically all tumors of this type. Whether this form of cell division is concerned in the growth of these tumors cannot be ascertained on account of the fact that their internal situation prevents observation during life. Certain peculiarities of nuclear structure are prominent in the epithelium. Many nuclei contain large globules of homogeneous eosin-staining substance, or there may be aggregations of small granules of similar material. Such nuclear structures are not confined to these tumors, but are found quite as frequently in the bronchial epithelium and also in the liver cells of the mouse.

Epidermoid carcinoma of the lung. — In several cases there was a diffuse growth of irregularly arranged epithelium throughout portions of the lung. Such epithelium occurred in the form of irregular masses or layers of flattened cells situated within spaces in loose connective tissue. From its character and its arrangement as stratified squamous epithelium, it is practically indistinguishable from epidermal tissue. Certain masses of epithelium present the characteristic germinative, spiny, granular, and horny layers of the epidermis. Intercellular bridges are, however, but slightly developed. That the epithelial proliferation was preceded by an inflammatory process is indicated by an increase in connective tissue throughout a portion of the lung much greater than that occupied by the tumor. The latter condition is usually associated with more or less modification of the bronchial and alveolar epithelium. It thus seems probable that the modification of the epithelium associated with extensive chronic inflammatory processes may in some way be concerned in the origin of lung tumors of this type. In this respect the latter are somewhat analogous to the cancers which follow chronic inflammatory changes in the skin, — X-ray, paraffin cancers, etc. Large crystals are often found within spaces lined by the irregularly growing epithelium. Minute crystals of similar character are found in large phagocytic cells throughout the lung. In only one of the four or five of these epidermoid cancers were mitotic figures found, but in this one they were numerous. This condition of diffuse carcinomatosis may co-exist with adenoma nodules, and there are all transitions between gland-like structures and the more irregularly growing stratified epithelium. The bronchial epithelium is in places irregular in its arrangement and stratified rather than columnar so that it is difficult to distinguish it from the epithelium of the tumor.

The absence in most of the cases of any tumor elsewhere in the body would indicate that these tumors are primary in the lung. Since the bronchial epithelium under certain conditions becomes stratified and, since the epithelium of the cystadenomata of the lung frequently shows a tendency to cornify, the development of primary squamous-cell carcinoma in the lung is not remarkable. Metaplasia as exemplified by the transformation of columnar epithelium into squamous epithelium has been repeatedly noted in the human being, both in the case of the endometrium and in the epithelium lining the gall bladder.

A minute nodule presenting all the structural features of a sebaceous cyst was found in the cecum of a mouse kept in this laboratory. A very good example of the metaplasia of epithelium is to be found in the gall bladder and the cystic and common ducts of the muskrat (Fiber zibethecus) as the result of the presence of liver flukes which have been found in about fifty per cent of these animals in the vicinity of Boston. The epithelium of the gall bladder and ducts is normally of the simple columnar variety in this species, but as the result of the long-continued presence of these parasitic worms the epithelium becomes modified to such an extent that it would readily be mistaken for epidermis (Figs. 1, 2, and 3). There is nothing to show whether this change in the character of the epithelium is due to the direct influence of the worms or to the inflammatory processes which they have set up in the subjacent connective tissue. In places, irregular processes of epithelium extend into the latter.

Such undoubted examples of the transformation of columnar epithelium into stratified squamous epithelium as the result of chronic injury may make more intelligible the origin of squamous-cell carcinoma from the pulmonary or bronchial epithelium.

Chronic inflammatory processes are also often found in association with primary cystadenoma of the lung. There may be an increase in the connective tissue with thickening of the alveolar walls, or changes in the bronchi associated with crystals which act as foreign bodies. In the latter case the bronchial epithelium is hyperplastic, the longitudinal folds are irregular, and irregular papillary projections are formed which sometimes extend for some distance along the tube. The epithelial cells are devoid of cilia and appear large and swollen. They often become detached and lie free in the bronchus. Mitotic division sometimes occurs in such detached cells. This suggests the possibility that the tumors which are situated apart from the bronchi and often beneath the pleura may arise from transplanted bronchial epithelium. There is little doubt that the epithelium of the established tumors is transplanted in this way. This form of metastasis may not, however, account for all multiple tumors of the lung, for there is no reason to assume that multiple primary tumors of similar type never occur in a single organ. The tumors of the lung are usually associated with large numbers of phagocytic cells loaded with slender crystals which are scattered throughout the uninvolved portion of the lung. The crystals appear to be of the same nature as the larger ones situated in the bronchi. They are sometimes found within the tumor epithelium. In the lung in one case of the series there is, in addition to a definite tumor nodule, an irregular proliferation of the epithelium of nearly all the small bronchi. This appears to have a definite relation to the presence of large crystals. It is possible that the chronic inflammatory changes which are often associated with large numbers of crystals may have some causal relation to the development of these tumors. These conditions are found, however, in mice without tumors. The occasional occurrence of a disintegrating worm or other animal parasite in lungs in which there is a tumor may be considered as coincidence just as are different forms of bacterial infections which are occasionally met with.

Notwithstanding the frequent association of changes in the connective tissue of the skin brought about by chronic inflammation or other processes with the development of epidermoid cancers in human beings, — similar chronic processes in the lungs of this species, such as are found in cases of marked anthracosis, rarely result in the development of tumors. The mere presence of chronic changes in the connective tissue, therefore, does not satisfactorily account for the biological modification of the epithelium which appears to be characteristic of epithelial tumors. Proof of the biological modification of tumor epithelium is found in the ability of tumor epithelium to invade normal tissues, to grow when transplanted to distant parts of the body as in the case of metastasis, and in the ability of the inoculable tumors to grow indefinitely when implanted on the tissues of young normal animals. Normal epithelium transplanted experimentally under similar conditions has been found to differ essentially in its biological properties from tumor epithelium. This holds true for connective tissue as well as for epithelium.

Lymphoma. — This tumor was first described in mice by Haaland[8] as a generalized hyperplasia of the lymphoid tissue throughout the body. From the occurrence of this condition in five of the mice kept in the cage in which the first case had been placed, it was thought to be possibly due to an infection of some sort. Inoculation of normal animals resulted negatively. I[4] subsequently described two cases, in one of which there was a single nodule in the inguinal region, while in the other there was a large mass in the mediastinum with secondary nodules in the pericardium and lungs. The conclusion was reached that we have to do in these cases with tumors of local origin, which may, however, extend to or metastasize to other portions of the body. There was no generalized hyperplasia of the lymphoid tissue. The inoculation of seventeen mice with one of these tumors resulted negatively. Murray[6] has recently reported four cases in two of which the growth was localized, while in the other two general dissemination had occurred. The view that the nodules in these cases are of the nature of new growths rather than of hyperplastic lymphoid tissue appears to have been accepted by him.

In the series here reported are included tumors composed of cells which are not concerned in the production of intercellular structures, and which resemble closely the cells of the lymphocyte series (Figs. 8–15). In absence of specific granules or fibrils it is difficult to determine the nature of a tumor from the morphological character of its cells, and it may eventually be found necessary to reclassify certain of the tumors included in this series. The cells may be of uniform character throughout a given tumor or they may vary considerably. The most common type of cell represented in these tumors is that of the cells of the germinative areas of the lymph follicles. Just as the latter cells vary considerably in size and morphology so do the tumor cells vary. They are usually considerably larger than the mature lymphocyte, and possess a relatively small amount of basophilic cytoplasm. Where cells are closely packed they appear flattened or angular in outline. The cell boundary is usually indistinct. In one tumor there was great variation in the size of the cells, the smallest being of the size of the lymphocytes of the circulating blood, the largest being of the size of the bone-marrow giant cells (Fig. 10). Variable numbers of large phagocytic endothelial cells occur in these tumors, in some of which they are limited to certain portions of the tumor often about necrotic areas, whereas in others they are generally distributed. Lymphomata in mice usually possess little supporting tissue so that they are of soft consistence. They are permeated by slender blood vessels most of which are of the size of capillaries. Most of the tumors of this series possess well defined lymph vessels which in many instances have the form of large irregular spaces. These vessels are usually filled with cells, which are smaller than the cells throughout solid portions of the tumor, and resemble more closely the small adult lymphocyte.

Tumors of this type were present in ten cases. The site of the primary tumor was the inguinal lymph node in two cases, the thymus in four, the mesentery in one, and the site of origin was not determined in three cases since the tumor had become so disseminated throughout the mesenteric lymph nodes and certain organs. The condition found in the latter cases could not, however, be interpreted as one of general hyperplasia of the lymphoid tissue, for it was invariably confined to some portion of the lymphatic system while other adjacent portions were uninvolved. The picture presented by most of the cases was that of a tumor having its origin at a certain point in the lymphatic system, and extending to or metastasizing to other organs just as is the case with other malignant tumors. In the four lymphomata which arose in the mediastinum, apparently in the thymus, the tumor tissue had extended along the perivascular lymphatics of the larger blood vessels into the lung in three, there were secondary nodules in the pericardium in all, and the myocardium was invaded in two cases. In other cases of the series the spleen, liver, kidney, pancreas, ovary, and omentum were infiltrated, and secondary nodules were found in the wall of the stomach and in the bone-marrow. There is also often a diffuse infiltration of fat tissue with the tumor cells.

In those cases in which the tumor had its origin in a lymph node the latter is entirely replaced, and no trace of its structure remains. In lymph nodes involved secondarily it is often possible to distinguish the tumor cells from the cells of the lymph node, and the structure of the latter persists for a time. In one case only were metastases absent. In this instance there was a large tumor in the inguinal region, while the other lymph nodes were not enlarged and on histological study were found to be not involved. The few cases in which there was involvement of a portion of the lymphatic system without there being present any larger nodule which could be interpreted as the primary growth may well be explained as a very early dissemination of the tumor cells so that the primary and secondary growths would show no marked difference in size. The distribution of the tumor tissue in these cases is more readily accounted for by extension and metastasis from a single primary tumor than by the development of multiple primary tumors. The origin of the nodules which occur in the heart and pericardium are certainly more readily accounted for by the transplantation of cells from a primary tumor of the thymus than by the hyperplasia of lymphatic tissue preëxisting at the site of these nodules. Difficulty may be found in distinguishing tumor from lymphoid tissue especially when one closely resembles the other or when normal tissue is invaded only to a slight extent by the tumor. From the fact that by present methods we are unable to distinguish tumor cells from normal cells of similar type, we are not justified in assuming that the normal cells are constantly being incorporated into the structure of these tumors. There is no indication in any of the cases of this series that the secondary tumor nodules arise through hyperplasia of the normal lymphoid tissue involved, while there is ample ground for the view that the lymphomata arise as local growths, and that the lymphoid tissue and various organs become secondarily involved by the metastasis of tumor cells.

In the primary lymphomata of the thymus epithelial structures are found. In one case there are irregular masses and islands of epithelium which in places shows a tendency to become stratified. In the small masses the cells are somewhat flattened and show a more or less concentric arrangement after the manner of Hassall's corpuscles. The centers of some such masses are degenerated, so that a space containing cell detritus is formed. Another type of epithelial arrangement is found in isolated gland-like structures consisting of a single layer of cuboidal or columnar epithelium enclosing a lumen. Ciliated epithelium is also found lining spaces (Fig. 12). Such structures, noted in the thymus by Remak, have been interpreted as the vestigeal remains of the entodermic anlage of this organ, in which the lumen has persisted. These epithelial structures are present only in limited areas of the primary tumors of the thymus, are not found in metastases, and show no evidence of continuous growth. They are not, therefore, to be regarded as an integral part of the tumor, but as belonging rather to the organ in which it has developed. There is no evidence of transformation either of the epithelium into tumor cells or of tumor cells into epithelium.

Lymphatic leukemia. — In one case in which the primary lymphoma was situated in the mesentery, there was an associated lymphatic leukemia. The blood was not examined during life, but in the study of stained sections of various organs large numbers of cells morphologically identical with the tumor cells are found distributed generally throughout the circulation.

The cells are approximately spherical, possess little cytoplasm, and are considerably larger than the normal lymphocytes of the circulating blood. In several other cases of the series, veins in intimate relation to tumor tissue are found filled with tumor cells, but since these cells are not found distributed throughout the circulation, it seems probable that this condition is of the nature of a terminal invasion of the vascular system by the tumor tissue, which possibly occurred only a short time prior to death. In those cases in which the tumor has infiltrated the liver, the cells are distributed between the liver trabeculæ apparently to a certain extent within the sinusoids, so that the picture is very much like that presented by the liver in leukemia. It is proposed to describe these tumors in more detail later on in connection with a more extended study of leukemia.

Unclassified tumors. — On account of the peculiar type of tissue growth found in the following three cases, and on account of the inability to determine the exact nature of the tissue in question, they are given somewhat in detail:

Case No. I. (1652). — A brown female mouse, age 1 year, 11½ months. Inoculated one year, seven months previously with an inoculable adenocarcinoma of mammary origin with negative result. Attention had been directed to the mouse on account of great enlargement of the belly. A blood smear showed no notable increase in white cells. The animal was killed and the tissues fixed at once.

The peritoneal cavity is dilated with a large amount of blood-tinged fluid. The liver is greatly enlarged. A portion of the left lateral lobe is of reddish color, but the greater portion of the liver is dull grayish yellow and opaque. The spleen is considerably enlarged, and there is a rounded swelling at one end. On the dorsal surface of the anterior lobe of the left lung is a grayish opalescent nodule three millimeters in diameter. The other organs appear normal.

The nodule in the lung is found to be of the nature of a primary papillary cystadenoma showing evidence of active growth in certain portions. Mitotic figures are present, although not numerous. In places the tumor epithelium is growing into the air spaces of the lung, and at one point it appears to be situated within a lymphatic. In the bronchi of this lobe of the lung are numerous large flat crystals which are stained dark red. The bronchi are slightly dilated and in many places the bronchial epithelium is modified, the cells are larger than normal, are devoid of cilia, and possess large pale nuclei. Mitotic figures are numerous and are present in cells lying free in the bronchus. The proliferation of the bronchial epithelium has produced in places long papillary projections which in some instances extend for a considerable distance along the bronchus.

From the study of sections taken through the enlarged end of the spleen, this portion of the organ is found to be replaced by tissue composed of cells with a relatively large amount of cytoplasm coloring pink or pale purple with the eosin methylene blue stain. The nuclei are large and hollow in appearance. They are often indented, and a definite centrosome and attraction sphere is usually apparent in the cytoplasm at this point. The chromatin is arranged in one or several coarse masses within the nucleus. Mitotic figures are numerous. This tissue appears to have grown out from the hylus of the spleen where it is found occluding the splenic vein. The cells of this tissue are closely packed and appear in some places in more or less continuous masses, in others disassociated from one another. They are readily distinguished from the cells normally found in the spleen, not only by their greater size, but also by their morphological character. They show considerable phagocytic activity and often contain fragments of red blood corpuscles and other cells.

Certain lobes of the liver are found to be necrotic with the exception of a few islands of living tissue around the portal canals. In other portions large necrotic areas are found having at their periphery a zone of congestion and hemorrhage. Around such areas there is thrombosis of the sinusoids. There is very slight inflammatory reaction in places. There are also minute sharply demarcated foci of coagulation necrosis. The cause of the injury to the liver tissue is found in the occlusion of the portal veins with tissue similar to that found growing in the spleen. This tissue in many instances is arranged in a thick irregular layer within the walls of the vessels. This layer is from three to six cells in thickness and appears irregular and broken in places. Certain veins contain only disassociated cells or groups of cells. The tissue is also found in the sinusoids and growing diffusely in the liver. There is thrombosis of certain of the veins as well as the sinusoids in association with the growth of this tissue.

In the portal veins the cells are on the average smaller and less uniform in size than those found in the spleen. Mitotic figures are frequent, but are often markedly atypical. It is probable that this difference in the character of the cells is due to faulty nutrition. Many of the cells show evidences of degeneration. The cells of the tissue growing in the sinusoids and diffusely through the liver are on the average considerably larger and resemble more closely those of the spleen. There is an increase in collagen fibrils in certain portions of the liver invaded by this tissue. Tissue similar to that found in the spleen occurs in portions of the omentum, and there are many large rounded cells of similar character growing on the surface of the peritoneum. The tumor cells are also found in the veins of the lung, pancreas, and kidney. The arteries, however, appear to be free from such cells.

The atypical tissue found in the liver and spleen of this case is evidently of the nature of a tumor (Figs. 16 and 17). Whether it represents a metastasis of the primary cystadenoma of the lung or a tumor of other origin, which has become generally disseminated, appears to be open to question. Since it has the general appearance of a carcinoma it might be thought that it was secondary to the cystadenoma of the lung. The latter tumor was, however, a small sharply circumscribed tumor showing evidence of growth only in certain portions. It is possible that a few of the cells of such a tumor on being transplanted to other organs assume greater activity of growth, and become otherwise modified both as to the morphology of the individual cells and as to their relation to the supporting tissues. In none of the fifty-one other tumors of the lung was there any evidence of metastasis to other organs. Not only are the individual cells morphologically unlike the epithelial cells of the lung tumor, but the tissue as a whole differs in its mode of growth and in its relation to other tissues from the epithelium of this and other similar tumors of the lung. In the latter the epithelium is found growing in a single layer which is invariably supported by connective tissue.

The tumor in the liver and spleen of the present case is composed of cells which cannot be identified with any degree of certainty with those of any normal tissue of the mouse. It grows profusely in the blood vessels and elsewhere without supporting tissue. In the larger blood vessels it tends to take the form of a thick uneven layer of cells lying against the intima. Large numbers of disassociated tumor cells are found in many of the capillaries and veins throughout the body, and it is evident that they are derived primarily from the great masses of proliferating tumor tissue in the liver and spleen. Their presence in the veins and not in the arteries is possibly explained by a slowing of the blood current in certain veins, the capillary branches of which have been more or less occluded by the tumor cells.

The indentation of the nuclei of these cells and presence of demonstrable centrosomes, associated with a tendency of the tissue to grow in a more or less continuous layer within the veins suggests that we have to deal with a tumor of endothelial origin. The presence of large numbers of tumor cells distributed quite generally throughout the veins simulates rather closely a leukemia. The fact that several other cases have occurred in which tumor tissue of similar character is found growing in the liver, spleen, and throughout the venous system, without any demonstrable tumor of the lung, makes it appear that there are in this case two tumors of different character.

CASE No. II. (1833). — An old brown mouse, sex not noted and age unknown. It had been observed to be sickly for some time before it was found dead. The liver is very large and evidently many times its normal volume. Its surface is uneven and granular and mottled with dull yellowish areas. The spleen is also very large and yellowish areas are apparent in its surface near one extremity. No other macroscopic changes are noted.

The liver is infiltrated throughout with tissue growing diffusely between the trabeculæ, which are widely separated, irregular and greatly attenuated in places. The tissue growing between the lobules is also found within the large veins. There are small areas of necrosis, most numerous near the surface of the liver. Small portions of the liver substance have apparently been replaced by connective tissue, and it is by the contraction of this that the irregularity of the surface of the organ has been produced. On account of imperfect preservation it is impossible to acquire as accurate a conception of the morphology of the cells as is desirable. They appear, however, as disassociated cells with pink stained cytoplasm and a vesicular eccentrically situated nucleus. There is often slight indentation of the nucleus, and a centrosome may be distinguished in many. In the veins the cells do not form continuous masses, but usually lie free. Masses of tissue of this type are found throughout the spleen so that the normal elements of this organ form a very small proportion of its bulk. In both the spleen and the liver there is considerable leucocytogenic activity. Large numbers of cells of the myelocyte type are found in certain areas, and they are often intermingled with the tumor tissue just described. They are distinguished from the cells of the latter by their smaller size, by the basophilic property of their cytoplasm, by the frequent ring-shaped nucleus, and in some cases by demonstrable specific granules. Atypical cells are found in the veins of the lung similar to those in the spleen and liver. They are not distributed uniformly throughout the blood, but occur in clumps which are often adherent to the vessel wall. In many areas the capillaries present an injection of these cells alone. Many of the smaller veins are occluded. In certain instances groups of atypical cells lying against the vessel wall are covered over with endothelium. Whether they have grown beneath the endothelium or the latter has grown over such attached masses, it is difficult to conclude. They are also found in the perivascular lymphatics. The arteries are nowhere involved in the growth of this tissue. The branches of the renal vein contain large numbers of the tumor cells. Masses of them are found in the glomeruli. In the veins of the kidney they are not attached to any extent, but lie free and intermingled with the blood. Centrosomes are readily distinguished. The appearance is very much like that of a leukemia. In sections of the heart the blood contains a few of the tumor cells and these together with the leucocytes are distributed uniformly among the red blood corpuscles.

In this case there is a growth of tissue in the spleen and liver which presents all the characteristics of a malignant tumor. The vascular system is also involved, the tumor cells being found in large numbers in the veins and capillaries. The picture presented by many of the veins is that of a leukemia (Fig. 18). The cells are not generally distributed throughout the blood, however, but are confined chiefly to the veins and capillaries. In many of the veins they are not evenly distributed, but occur in clumps and are often adherent to the wall of the vessel. The perivascular lymphatics are also invaded. The exact genetic position of the tumor cells has not been determined, but it seems probable that they are derived either from endothelial cells or from primitive cells of the myelocyte type. Although the cells are considerably smaller, they resemble rather closely those found in the spleen and liver in the preceding case. It must also be left undecided whether the condition found in the blood in this case is essentially one of leukemia.

CASE III. (2522). — An uninoculated male albino mouse evidently very old, found dead. At autopsy there is marked subcutaneous edema in the region of the axillæ and the flanks, so that when the skin is dissected back a thin yellow tinged fluid flows away. Both axillary and inguinal lymph nodes are much enlarged, and when incised considerable fluid escapes. The cut surface shows in part a soft opaque whitish tissue intermingled with more or less blood-colored fluid. The peritoneal cavity is distended with thin blood-tinged fluid. Portions of the peritoneal surface of the spleen and the kidney present minute slightly roughened elevations. Throughout the lungs there are minute dark specks. The liver is much enlarged. Its surface is somewhat uneven, and presents a mottled appearance from the presence of opaque whitish and dark red areas. The most extensive changes are in the left lateral lobe. The spleen is not notably enlarged, but is soft and flabby and of pale brownish red color. On section a soft, dull red mush oozes out. The kidneys appear normal. The bone-marrow is red throughout. Post-peritoneal and thoracic lymph nodes not enlarged.

On histological examination both axillary and inguinal lymph nodes are found to be almost wholly replaced by an atypical tissue evidently constituting a tumor. These masses are to a large extent necrotic, and into such portions hemorrhage has apparently taken place. In the necrotic portions small islands of tumor tissue situated around blood vessels have survived. In places where the structure of the lymph node is still distinguishable, the lymph sinuses are dilated with masses of the tumor tissue. The tumor is composed of cells of large and fairly uniform size. There is usually one, though rarely there are two or more, rounded vesicular nuclei with several large deeply stained masses of chromatin. There is a relatively large amount of cytoplasm which is invariably loaded with globules that take a purplish color by the eosin methylene blue method. These globules vary greatly in size from barely visible granules to coarse masses about the diameter of a red blood corpuscle. There is a clear space in the cytoplasm near the nucleus of these cells similar to that surrounding the centrosome in plasma cells. The cells of this tumor do not appear to form continuous masses, but to a large extent lie free from one another. Where they are closely packed they are angular in outline, but are otherwise commonly rounded. No mitotic figures are found. The lung is apparently not involved to any great extent, although cells of the character of tumor cells are found here and there in the capillaries. Those portions of the liver from which sections were taken are infiltrated throughout with tumor tissue, similar to that found replacing the axillary and inguinal lymph nodes, and fully equal in amount to the liver tissue proper. Molds of tumor tissue are found in the branches of the portal vein and there is also a more or less diffuse growth between the trabeculæ. There are large irregular areas of necrosis in the liver, probably produced by interference with the blood supply. One tumor cell was found in mitotic division. The spleen is for the most part replaced by tumor tissue of the character of that already described. There is extensive thrombosis in the venous sinuses, associated with moderate numbers of polynuclear leucocytes. The blood vessels of the bone marrow of the femur contain many tumor cells, but the tumor has not developed to any extent in this tissue. Tumor cells are present in large numbers in both arteries and veins of the kidney and in the glomeruli.

In this case (see Figs. 19 and 20), as well as the two preceding, the tumor cells are generally disseminated throughout the circulation, so that in this respect the condition of leukemia is closely simulated. Unfortunately in the first case only was the blood examined during life, and in this case blood from the tail vein showed no atypical cells. The tumors in these three cases had attained their greatest development in the spleen and liver, although in the third case there were nodules of considerable size in the axillary and inguinal regions. Another feature common to the three tumors is the prominence of the centrosome. The tumor cells, however, differ considerably in the three cases, and, since they are unassociated with any specific intercellular substance and lack any characteristic arrangement, they are left unclassified. In the third case (No. 2522) the tumor cells possess cytoplasmic granules suggesting that they are of the nature of myelocytes. There is, however, no uniformity in the size of these granules, and the material found in fine granules is also found in coarse globules, some of which are larger than red blood corpuscles. It is possible that with further study and with the detection of such cases during life, the nature of the tumors may be ascertained.

Tumors of the mammary gland. — The epithelial tumors which occur in the region of the mammary gland of the mouse have been frequently propagated by transplantation from mouse to mouse, and have thus furnished the basis of a great part of recent tumor investigation. They have been most fully classified by Apolant,[7] who recognizes the following types: adenoma simplex, cystadenoma simplex, adenoma cysticum œdematosum seu hæmorrhagicum, cystadenoma papillare, as well as carcinoma simplex alveolare, cystocarcinoma hæmorrhagicum, and carcinoma papillare including the so-called fissure-forming cancer. Apolant states that various portions of a single tumor often show great structural differences, so that many of the above types are represented in a single tumor.

Since the structural character of these tumors is frequently such as to present all transitions between simple glandular tumors and irregularly growing carcinomata, he takes this as evidence of the origin, in many cases, of the latter in adenomata. Murray,[8] however, believes that in certain tumors at least the reverse is true, — that solid alveolar masses of tumor epithelium become subdivided and differentiate into structures more or less glandular in character. In the course of the continuous transplantation of tumors Apolant found that tumors which had been previously alveolar in several instances became glandular. This transformation he believed to be due possibly to the fact that the mice in which it took place had been partially immunized by previous injections of mouse blood. Thus not only may glandular epithelium revert to a more primitive type as found in alveolar or irregularly growing carcinomata (anaplasia of von Hansemann), but the epithelium of such tumors is found to be capable in certain instances of a considerable degree of differentiation.

In addition to the subcutaneous epithelial tumors of the types enumerated by Apolant a number of tumors have been described which are apparently not included in his classification. A tumor the epithelium of which has in part the structural arrangement of gland acini, in part the characteristic features of the sebaceous follicles, and which is elsewhere intimately intermingled with connective tissue, has been found in a Japanese waltzing mouse. A detailed description of this tumor has already been given, and the results of its propagation are to be found in the paper following. The original tumor appears to be of a somewhat different type from the sebaceous adenomata recently described by Murray. Loeb[9,10] had previously described a tumor of similar character, which occurred in a Japanese waltzing mouse. Loeb's tumor as well as my own was readily propagated in the variety of mice in which it arose. Certain of the tumors which resulted from the transplantation of Loeb's tumor into normal animals consisted largely of connective tissue, so that nodules of both mixed tumor and sarcoma were produced. Curiously enough one of the four mice into which the Japanese waltzing mouse tumor of this laboratory was first inoculated developed a nodule which consisted in part of atypical connective tissue and in part of glands (Figs. 28 and 29). This nodule which had attained only the size of a small pea in slightly over nine months of growth was not inoculated into other mice. In no other tumors of this strain has any sarcomatous element appeared during two and a half years of propagation. Since the original tumor presented various types of epithelial arrangement, and, since its transplantation resulted in a tumor which consisted in part of connective tissue, it appears not improbable that it was a mixed tumor with which we had to deal. It could not, however, be ascertained in this single instance whether the sarcomatous element was derived from the tumor tissue implanted or from the tissues of the host.

Another type of mouse tumor not included in Apolant's classification is the squamous-cell carcinoma. Murray describes three such tumors which occurred in the neck, the axilla, and about one of the nipples respectively. In the following case a tumor which occurred in the region of the external genitalia presented a marked degree of keratinization.

CASE No. IV. (2159).— A black female mouse, age 1 year, 6 1/3 months. A tumor situated to the right of the external genitalia, is approximately spherical in form, and measures 1.5 centimeters in diameter. On section its cut surface presents rather coarse subdivisions or lobules. In the central portion the surface shows minute yellowish streaks and points. There are small whitish nodules in the anterior and middle lobes of the right lung, and in the anterior lobe of the left lung. The other organs appear normal.

The subcutaneous tumor consists of epithelium which varies considerably in its arrangement, associated with a very small amount of stroma. The epithelium is in part arranged in solid alveoli, some of which show a tendency to central necrosis and softening, but by far the greater number present masses of desquamated epithelium in the form of thin concentrically arranged lamellæ. Between the alveoli there are irregular duct-like structures which contain eosin-staining coagulum. Indefinite gland-like and duct-like structures are also found in many of the more solid masses of epithelium. The lungs present multiple rounded nodules of papillary cystadenomata of the type which are frequently found in the lungs. There are also irregular masses of flattened epithelium scattered diffusely throughout a portion of the lung. It appears to be growing within air spaces, and bears no specific relation to blood vessels or lymphatics. In one area all transitions are found between the adenoma type of structure and the more irregularly growing epithelium. Since similar primary tumors of the lung have been found in many other cases, this tumor is evidently primary and distinct from the subcutaneous tumor.

In this case the subcutaneous tumor consists largely of concentric whorls of flattened epithelial cells (Fig. 22). The epithelium otherwise has either an alveolar or a glandular arrangement. All intermediate forms of structural arrangement are found, and it appears probable that both glands and keratinized portions are modifications of a single mass of epithelium. The histological structure of the tumor indicates the keratinization of epithelium which has otherwise a more or less glandular arrangement. A tumor of similar character, which was propagated by Murray, maintained an alveolar structure with more or less keratinization for several generations, but subsequently became in some instances glandular in structure.

Another tumor (Case No. V. — 2404) in which there was a great deal of hemorrhage is included in this series of mammary tumors, although it is of an unusual type. It occurred in a black female mouse nearly two years old. The tumor appeared as a dark purplish swelling, which did not increase appreciably in size during ten days for which it was under observation. On section the mass was found to consist of tissue, composed of atypical cells without characteristic arrangement, and blood situated in large and small irregular spaces, most of which are not lined with endothelium. Both old and recent thrombi were found. The tissue of which a large portion of the nodule is composed consists of cells varying greatly in size and morphology. Some of those of great size have lobulated or multiple nuclei, and syncytium-like structures are also found. With Mallory's connective tissue stain, the cells appear to be enmeshed with a very delicate blue-stained reticulum. No fibroglia fibrils could be demonstrated, however, with the phosphotungstic acid hematoxylin stain. Where there are masses of closely packed cells, the contiguous cell membranes are distinct, and the tissue has the general appearance of epithelium. The lung in this case presented a minute cystadenoma. Although the cells of which the subcutaneous tumor in this case is composed present no distinguishing features, it seems probable from its general character that it is to be classed with the hemorrhagic cancers.

In the following table are data concerning all the epithelial tumors of subcutaneous origin which have appeared in this laboratory:

No.	Type of Tumor.	Site.	Metastases.	Results of Transplantation.
1415	Adenocarcinoma.	Inguinal.	Nodule near axilla.	Ten mice negative (tumor infected).
1489	Adenocarcinoma in Japanese waltzing mouse.	Anterior to right foreleg.	Small nodule in inguinal region.	Propagated to present time (2½ years).
1559	Cleft forming carcinoma.	Posterior to left foreleg.	Axillary lymph nodes.	Eight mice. Tumor developed in two.
1647	Cystocarcinoma hemorrhagicum.	Inguinal.	None.	Ten mice. Negative.
1361	Adenocarcinoma.	Axilla.	None.	Propagated for three generations.

No.	Type of Tumor.	Site.	Metastases.	Results of Transplantation.
2159	Adenocarcinoma with keratinization.	Near external genitalia.	None.	Not transplanted.
2216	Cystocarcinoma hemorrhagicum.	Axilla.	None.	Four mice negative.
2404	(Carcinoma?) hemorrhagicum.	Axilla.	None.	Not transplanted.

Portions of six of these eight tumors were inoculated into normal mice with the result that three grew. Only one, the Japanese waltzing mouse tumor, has been continuously propagated. In one of these eight cases there was metastasis to the neighboring lymph nodes, which were practically replaced by the tumor, and in two other cases there were, in addition to the tumor described, other small subcutaneous nodules of similar character, which were possibly of the nature of metastatic nodules, although it could not be proved that they were not distinct primary growths.

Since less than ten per cent of the tumors which have thus far occurred in this laboratory have been mammary tumors, it would appear either that there is some peculiar difference in the incidence of these tumors here as compared with other laboratories or that the internally situated tumors are quite generally overlooked by other investigators through failure to make thorough post-mortem examinations.

Hypernephroma. — Tumors of the kidney have been found in four cases, two of which were described in a previous paper as cystadenoma.

CASE No. VI. (1836). — An old gray female mouse. The left kidney presents at its posterior extremity a rounded yellowish nodule measuring 7 x 8 x 9 millimeters. This nodule consists of tissue, somewhat alveolar in its arrangement and composed of cells with small rounded nuclei and abundant eosin-staining cytoplasm. The cytoplasm of the cells throughout the nodule is vacuolated and the cell membranes are distinct. The nodule is vascular throughout, and contains relatively little connective tissue.

In this case there was also a papillary cystadenoma of the lung, a carcinoma of the ovary, and a lymphoma infiltrating both kidneys.

CASE No. VII. (1848). — An old female albino mouse. Near the posterior extremity of the right kidney is a somewhat opaque yellowish-gray nodule protruding from the surface, and measuring 2.2 x 2 x 1.5 millimeters. The right adrenal is situated in its usual position at the anterior extremity of the kidney. The liver is atrophied, and its lobules are small and rounded. Death was apparently due to pyloric obstruction from cotton which had been devoured.

The nodule consists of irregular rounded masses of tissue which is composed of large cells with small nearly spherical nuclei, and abundant cytoplasm staining red or slightly purplish with the eosin methylene blue stain. These cells stain much more deeply than those of the adrenal in the same section, but otherwise resemble them closely. The central portion of the nodule is necrotic, and the cells adjoining are vacuolated. Clefts possiby left by cholestrian crystals are found near the surface of the nodule overlying the necrotic portion.

From the resemblance of the cells which compose the tumor in these two cases to those of the adrenal, it is evident that they are to be regarded as hypernephromata (Fig. 23). The two tumors previously described should probably be similarly classified, although they present an irregular glandular arrangement of tissue.

Papillary cystadenoma of the ovary. — Papillary cystadenoma involving both ovaries has been observed by Jobling.[5] The tumors of the ovary found in the following four cases differed from one another in certain respects, but all were more or less papillary in character (Fig. 24).

CASE No. VIII. (2483). — A black female, age 1 year, 11 months, 24 days. In the place of the two ovaries are rounded nodules. The one on the right side is bi-lobed and measures 4 x 3 x 3 millimeters, and the one on the left side is tri-lobed and measures 9 millimeters in its greatest diameter. Both nodules consist of pinkish tissue, which to the naked eye is solid and homogeneous throughout.

In stained sections the nodules are found to consist in part of epithelium irregularly glandular in its arrangement, and in part of flattened epithelial cells intimately intermingled with the connective tissue. In certain portions thick papillary processes of epithelium extend into and along the lumina of the gland. These processes are of a peculiar sort in that they have no central core of connective tissue, but are composed wholly of epithelium which only here and there shows a loose attachment to the wall of the gland-like space in which they are situated. The epithelium of which they are composed is either high columnar with definite cell membrane or is in the form of an indefinite fused mass. There is a relatively large amount of cytoplasm which stains rather faintly, and the nuclei vary greatly in size. No mitotic figures are found, but masses of epithelium occur in the lymphatics, so that it is evident that the nodules are of the nature of malignant tumors. A portion of the ovary with a corpus luteum was found on the surface of one of the tumors.

In case No. V. (1836) in which one of the hypernephromata described above occurred, there was a small nodule, occupying the position of the ovary, which was found to be a tumor similar in many respects to those of the preceding case. The epithelium is, however, of low type and stains intensely. It is arranged in irregular gland-like structures or solid columns which are intimately intermingled with the connective tissue. On one surface of the nodule there are cysts into which extend papillary processes clothed with epithelium.

CASE NO. IX. (2551). — Mouse of Family A. Heredity series. Age 2 years, 3 months, 10 days. Death due to the growth of a large primary tumor of the lung. The right ovary is replaced by a mass of soft pinkish tissue about the size of a large pea. This consists of irregularly growing epithelium having in places a more or less glandular structure. There is no tendency to form spaces, so that the tumor is practically solid.

CASE NO. X. (2553). — Mouse of Family A. Heredity series. Age 2 years, 9 months, 18 days. Killed on being found in dying condition. Both ovaries replaced by masses of translucent whitish tissue measuring 5 x 3 x 3 millimeters. A portion of one of these tumors was inoculated into another mouse of the same lot, and in this animal there is at the present time a minute hard mass at the site of the inoculation.

One of these tumors is composed in part of pale staining epithelium, growing irregularly and in places forming thick papillary processes filling all available spaces. The remainder of this and the whole of the other tumor is composed of deeply staining epithelium having an irregular glandular arrangement. The cells of this portion are small as compared with that previously described.

The tumors in these four cases differ considerably from the more common types of ovarian tumor in the human being. They are composed of epithelium, more or less glandular in its arrangement, and showing but slight tendency to form cysts. The ovarian tumors were double in two of the four cases. Papillary ingrowths fill all available space within the gland-like structures, so that the tumor remains essentially solid. The epithelium of which these tumors are composed is of similar character to the peritoneal mesothelium found at the attachment of the ovary.

Giant-cell sarcoma of the orbit. — This tumor like several of those already described occurred in a family of mice derived from a tumor parent (Family A).

Case No. XI. (2502). — Black female, age 2 years, 1 month. A rounded mass measuring about 3 centimeters in diameter was found during life bulging from the right orbit and extending over the forehead toward the left eye. The skin over the mass was smooth and pink, and there was a crust at the site of the opening of the eyelids. When the skin was dissected back the mass was found to consist of firm pink tissue with the remains of the eye imbedded in its interior. The central portion underlying the crust appeared opaque and yellowish, and was apparently necrotic. Certain portions farthest from this were employed to inoculate eleven mice. The tissue was evidently infected, for sloughs followed in all but one of the eleven inoculated mice. In this mouse a mass appeared which increased gradually to the size of a pea, remained stationary for several weeks, and finally disappeared.

Histology: The tumor is composed of atypical cells which vary in size and shape. Nearly all are considerably larger than normal connective tissue cells, and many attain the proportions of giant cells. The large cells are often rounded or present thick rounded processes, while the smaller ones appear in sections spindle-shaped. Each cell possesses a relatively large amount of coarsely reticular or fibrillar cytoplasm. The nuclei contain large masses of deeply stained material, which in many cells appear globular and hyaline. The nuclei of the large cells are lobulated and irregular, and many cells are multinucleated. The cytoplasm of the large cells is generally differentiated into a blue staining outer layer of loose fluffy structure and an inner eosin-staining portion which appears finely granular. Nuclear figures are not infrequent. Associated with the tumor cells are straight rigid fibrils extending lengthwise over the surface of the cytoplasm. These are identical in every respect with the fibroglia fibrils of connective tissue cells.

The tumor is covered externally by the skin, but there is otherwise no limiting capsule. In the central portion of the tumor there are colonies of cocci in the vicinity of which there is suppuration. Internally the tumor has grown along the surface of the bone, and has penetrated the skull. Externally it has grown into a vein. The lung presents minute lens-shaped masses of tumor tissue, of similar character, at the pleural surface and smaller masses within its substance. Mitotic figures are more numerous in the metastatic nodules than in the primary growth.

The tumor in this case is composed of cells which possess certain characteristics of connective tissue cells and are, moreover, associated with fibroglia fibrils (Fig. 25). It has infiltrated the surrounding tissues, including blood vessels, has invaded the skull, and has become disseminated throughout the lung.

Fibro-sarcoma of the liver:

Case No. XII. (2219). — A white female, age 2½ years. The liver is greatly enlarged. The right lobe presents a thin-walled yellowish cyst, containing a worm, and apparently connected with the bile duct. Throughout the liver are small whitish nodules, many of which are sharply circumscribed and rounded. None exceeds 3 millimeters in diameter. The spleen is very large, its surface is puckered in places and presents several ill-defined whitish areas.

The nodules in the liver are found to be composed of cells of the nature of connective tissue cells, associated with collagen fibrils. These cells vary in size and shape, and are often elongated or irregular. Some cells are multinucleated. Others present a centrosome situated opposite an indentation of the nucleus. Scattered here and there throughout the tumor nodules are isolated degenerating liver cells and small bile ducts. The tumor has grown diffusely throughout the liver so that the liver columns are compressed. The spleen also presents several masses of atypical tissue such as that present in the liver. Few mitotic figures are present.

Death was due to bacterial infection causing miliary abscesses in the gastric mucosa, the lung, and the kidney. There was also a minute papillary cystadenoma in the lung.

This case presents a diffuse growth of sarcoma throughout the liver (Fig. 26), associated with a cysticercus cyst.

There is no nodule of such size that it can be considered the primary growth. The tumor presents no unusual features, and is apparently of the nature of a sarcoma (fibro-sarcoma).

In addition to the types of tumors already enumerated an adenocarcinoma of the small intestine has been described by Bashford, Murray, and Cramer,[11] and other tumors — namely, squamous-cell carcinoma of the stomach, adenoma of the liver, spindle-cell sarcoma of the region of the kidney, sebaceous adenoma, angioma, and chondro-osteosarcoma — by Murray.[6] The systematic investigation of the tumors of the mouse has served to indicate the great variety of tumor types in other than the human species. If it were possible to keep large numbers of the longer-lived animals under observation for sufficiently long periods it is probable that a correspondingly large number of different types of tumors would be found in many species.

The inoculation of tumor tissue into mice does not appear to affect appreciably the frequency of primary tumors. In a series of seventy mice in which primary tumors have developed, twenty-two had been previously inoculated and forty-eight were uninoculated. Of the total number of old mice kept in the laboratory, the inoculated usually outnumber the uninoculated. In a previous paper it was shown that immunity to an inoculated tumor does not ensure immunity from spontaneous tumors. The present data may possibly indicate that mice refractory to the inoculable tumors are relatively less liable to develop spontaneous tumors than mice in general, but such a difference, if it exists, is no more than a relative one.

Primary tumors occur less frequently in male as compared with female mice. In the seventy tumor-mice of the present series, only fourteen were males. In two of these the primary tumor was of the nature of a lymphoma, in one the tumor was a doubtful nature, and in the remaining eleven there were primary tumors of the lung.

There is no indication that any of the tumors of the present series are of the nature of congenital tumors. Only two tumors have been found in mice less than six months of age. One of these, a lymphoma of the thymus, caused death at the age of five and a half months, and the other, a papillary cystadenoma of the lung, was found in a mouse killed at the age of five and a half months. In the seventy tumor-mice of the present series twenty-six were old mice of unknown age, whereas either the exact or the approximate age of the other forty-four was known. Two of these presented tumors at an age less than six months, eleven at ages between six and twelve months, four between a year and a year and a half, twelve between a year and a half and two years, and fifteen between two and two and a half years. Since many times the number of tumors are found in mice which attain an age of one year or over as occur in those which die at an earlier age, it is apparent that the incidence of tumors in mice increases with age. An analysis of the present series shows no appreciable difference in the age incidence of the various types of the tumors found. There were only two sarcomata, however, and the age of the mice bearing lymphomata was known in only five cases. The mice in five other cases were apparently very old.

The frequent association of chronic inflammatory processes with tumors of the lung suggests that there is an etiological relationship between the two conditions. In many cases in which there are tumors in the lung there is a more or less general modification of the bronchial epithelium, chiefly of the nature of a hyperplasia with more or less irregularity of growth. Crystals which often attain considerable size are frequently found in the lungs of mice where they act as foreign bodies. Their presence is not, however, invariably associated with a hyperplastic condition of the epithelium. Nothing is known of the conditions under which these crystals are formed. Disintegrating worms have been found in the lungs in several cases in which there were lung tumors. There is always an inflammatory reaction on the part of the tissues around these parasites, but all tumors thus far found have been situated at a distance from the latter. Since these parasites are so much less frequent than the tumors of this organ, it is not proved that their occurrence is more than coincident. Bacterial infections have been found associated with tumors of the lung in as many cases as have parasitic worms. Disregarding the more specific agents, it appears probable, however, that chronic inflammatory conditions favor the development of tumors. The changes most frequently noted are the hyperplastic condition of the bronchial epithelium, the increase in the connective tissue, and the presence of crystals, of which those of large size are situated free in the air spaces, whereas the smaller ones are found within phagocytic cells. In those cases in which there was a diffusely growing squamous-cell cancer, the bronchial epithelium is generally modified, presents great irregularity of arrangement, and in places has the appearance of invading surrounding tissue. From these facts it appears probable that many of the primary tumors of the lung of the mouse are in certain respects analogous to the tumors developing subsequent to X-ray burns. Like the latter they are associated with chronic inflammatory processes, as the result of which the epithelium is quite generally modified throughout large portions of the lung, often to such an extent as to suggest the early development of carcinoma.

In a previous paper (Tyzzer[4]) the relative frequency of tumors in one family of mice was mentioned and attention was called to the method of breeding tumor-mice in order to determine the part played by heredity in the incidence of tumors. At that time observations had been completed on only one family of mice (Family C). In this family three of the twenty-four offspring presented papillary cystadenomata of the lung similar to the one found in the parent of the family. Sixteen per cent of this family, therefore, presented tumors, notwithstanding the fact that the ages attained by its members were not great, in no case being more than fourteen months.

The observations on Family B, which consisted of twenty-nine offspring of a female mouse with a lymphoma in the inguinal region, are now complete. Of the twenty-nine offspring, one developed diffusely growing lymphoma (Fig.

13), and one a papillary cystadenoma of the lung. Thus including the tumor parent only ten per cent of the total number developed tumors. Five of the twenty-nine mice died during the first six months, and only seven attained the age of one year. The offspring resulting from the further breeding of this family were used for inoculation purposes. In an unknown number of such as survived the experiments for which they were used, two developed primary tumors of the lung and one a hemorrhagic adenocarcinoma.

The family designated Family A in the paper already referred to consists of ninety-eight mice derived from the mating of a mouse which never developed a tumor with a female mouse in which there was a large cystadenoma of the lung. Including both parents and offspring there were thus one hundred mice. As soon as this number was reached the males were separated from the females and breeding discontinued. The entire family was then kept under observation, and the best possible conditions furnished them in order to prolong life as long as possible. A record was kept of each mouse, including description, date of birth, etc., and an autopsy was performed as soon as possible after death. Those which presented external tumors were killed as well as others which appeared to be in a dying condition. Thirty-three of the offspring died at ages less than six months of infections and various other causes. Many of the remaining sixty-five offspring attained great age, and the three which now survive are all more than two and a half years old. If those mice which died at ages less than six months are eliminated, there are then sixty-two which died after reaching full maturity. Twenty, or thirty-two per cent, of this number presented one or more tumors. Seventeen presented primary tumors of the lung, most of which were similar to the tumor which was found in the female mouse from which this family was derived. In one of these mice there was also a subcutaneous squamous-cell carcinoma, in one an ovarian tumor, and in another there was also a subcutaneous hemorrhagic carcinoma. In one of the three remaining mice there was a giant-cell sarcoma of the orbit, and in the other two there were double papillary cystadenomata of the ovaries. The percentage given above may be slightly increased by the development of tumors in the three old mice which survive. The fact that over twenty per cent of the entire family (including the three mice now living and the thirty-three which died while young) developed tumors indicates that heredity may be a factor in the development of primary tumors.

The mice of this family may be classified as to whether they are the offspring of parents without tumors, or of parents one of which developed a tumor. The incidence of tumors in the two respective groups may then be compared. Unfortunately only one of this family is derived from parents both of which had tumors. The number of offspring of the various matings and the number of tumors which they developed are as follows:

Class I. — Offspring of parents neither of which had a tumor:

No. 3 mated with No. 4 = 5 offspring .... 0 developed tumors.
" 3 " " " 8 = 29 " .... 5 " "
" 15 " " " 16 = 16 " (one alive) ... 0 " "
" 19 " " " 21 = 11 " .... 2 " "
" 31 " " " 32 = 1 " .... 0 " "
" 34 " " " 37 = 6 " .... 2 " "

Total, 68 offspring (one alive) ... 9 developed tumors.

Tumors in 13 per cent of those which died.

Class II. — Offspring of parents one of which had a tumor:

No. 1 mated with No. 2 = 2 offspring .... 0 developed tumors.
" 3 " " " 13 = 9 " .... 4 " "
" 3 " " " 25 = 5 " .... 2 " "
" 22 " " " 24 = 7 " (one alive) ... 4 " "
" 36 " " " 38 = 6 " .... 1 " "

Total, 29 offspring (one alive) ...11 developed tumors.

Tumors in 39 per cent of those which died.

Class III. — Offspring of parents both of which had tumors:

No. 44 mated with No. 45 = 1 offspring (now alive).

In this family it is evident that tumors are about three times as frequent in the offspring of a tumor parent as in the offspring of parents without tumors.

A number of the mice of Class I. which were bred from succumbed before reaching the tumor age, so that they and their offspring might under other circumstances be included in Class II. On the other hand, many of the offspring of Class I. died while very young, so that it is necessary to consider the relative ages attained by the mice in the two classes. It was found that forty of the offspring of Class I. lived to an age exceeding six months, while twenty-four of Class II. survived this period. The estimates revised on this basis show tumors in twenty-three per cent of the mice in Class I. which died at ages greater than six months and tumors in fifty per cent of the mice in Class II. A further analysis with regard to the ages attained by the mice in the two respective classes shows that a larger proportion of those of Class II. developed tumors than those of similar age in Class I. Thus in nine mice of Class II., dying at ages exceeding two years, seven or seventy-seven per cent had tumors, while in six mice of similar age of Class I. three or fifty per cent developed tumors. The numbers dealt with here are too small to warrant conclusions. The data thus collected would tend to indicate, however, that there is greater liability to the development of tumors in the offspring of parents with tumors than in the offspring of parents which are throughout life free from tumors. These results are, however, of statistical nature, and larger numbers are desirable.

Two mice of Family A, which developed tumors, had been mated together, and their offspring are now living under observation.

The ground formerly taken by me that races of mice insusceptible to certain inoculable tumors were therefore to be considered less liable to spontaneous tumors is evidently not fully warranted. In Family D, which had been found insusceptible to the Jensen and Ehrlich stamme II. tumor, and was thus taken as a control with which to compare the three tumor families already discussed, one mouse has developed a papillary cystadenoma of the lung. Primary tumors have also developed in common mice which had been previously found insusceptible to the inoculable tumor of the Japanese waltzing mouse.

The incidence of tumors in certain families might be compared with the incidence of tumors in tame mice at large. Records have been kept of about eleven hundred mice in addition to those of the three "tumor families" already discussed. A large proportion of this number are of the same stock as the tumor families, so that the comparison of the two is of no great value. In fact a large proportion of the mice kept in stock were derived by the cross-mating of certain mice of the respective tumor families. A considerable number of the tumors discussed above developed in mice of such origin. Certain lots of mice that were purchased consisted chiefly of old females which had previously been used for breeding, and these furnished many of the tumors. These were from the same source as the tumor females from which Families A and B were obtained. Nothing was known of their age beyond that they were old mice. Apart from the purchased mice there were autopsied somewhat over seven hundred mice whose ages were known. Few of this number, however, lived to be a year old, and, since most of the tumors in the tumor families developed at an even greater age, there is little value in comparing the incidence of tumors in the two. It is questionable whether an investigation so essentially statistical in character warrants the time and labor necessary to keep the required records of large numbers of animals under observation for long periods of time. In continuing the investigation of the part played by inheritance in tumors, in place of attempting to keep records of a large number of mice of the same and mixed stocks, the tumor family will be compared with a family of another origin. A number of mice have already been obtained from parents both of which developed tumors.

The results obtained thus far go to show the frequency of tumors in certain families of mice. It would be premature to conclude from the data collected that the development of a tumor is based upon the inheritance of a specific character. It appears probable that certain inherited characters may become apparent only late in life during the period of the decadence of the individual. Moreover, such characters may possibly become apparent only under certain conditions. Racial differences are found in animals (and in plants as well) with regard to their susceptibility to pathogenic organisms, but this peculiarity becomes apparent only in the presence of the latter. The importance of certain peculiar injurious agents in the etiology of certain tumors can no longer be questioned. The X-ray is probably the most familiar example of these. The changes in the skin in old age simulate in certain respects the changes associated with these tumors, and it is probable that the conditions are analogous. It is questionable, however, whether such conditions are associated with other types of tumors. In the series of tumors in this paper many of the primary tumors of the lung were associated with chronic inflammatory changes and with foreign bodies in the form of large crystals situated in the bronchi. Age is also an important factor in the development of tumors in mice. The percentage of tumors in the mice of Family A over two years of age is such as to lead to the conclusion that, with the members of this family that do not succumb to infectious diseases and their sequellæ, cancer is almost inevitable.

In a recent paper by Bashford,[12] the possible influence of heredity in the development of cancer is discussed, and attention is called to the experimental method in re-approaching this subject. He also has adopted the method of breeding from cancerous mice and subsequently noting the incidence of cancer in the offspring. Mice with carcinoma of the mammary gland were used. He states "that thus far there is no evidence that the liability to carcinoma has been enhanced by systematic in-breeding."

The investigation of heredity with reference to cancer may be objected to on the ground that it affords no alleviation for this condition. Whether it is found to be of great or of slight importance in the etiology of cancer we should not shut our eyes to the facts of the case. The results of the present investigation indicate that one of the factors in the development of tumors is to be found in an inherited character or peculiarity.

SUMMARY.

Of the tumors which have occurred in the mice kept under observation the greater proportion have been internally situated tumors. It seems probable that many such tumors are overlooked, while the external tumors are usually conspicuous enough to attract attention.

The number of tumors found is relatively large with respect to the number of mice kept under observation. This may possibly be due to a peculiarity of the stock, but more probably due to the fact that the mice are kept under conditions which contribute to longevity, and on account of the practice of performing an autopsy on each animal which dies, with a microscopical examination of every suspicious nodule.

Primary tumors of the lung appear to be by far the most frequent tumors of mice. These lung tumors are of two types, the papillary cystadenoma and the epidermoid carcinoma. The papillary cystadenoma frequently attains a size sufficient to cause death. Its growth may be expansive or infiltrative in character. The bronchi are not infrequently invaded, and tumor cells which become free in the air spaces may be transported to other portions of the lung where they give rise to secondary tumor nodules. In one case the liver and spleen were infiltrated with tumor tissue which may possibly have been derived from a primary tumor of the lung, but, apart from this, metastases were not found in other organs than the lung. The epidermoid cancers consist of diffusely growing epithelium which shows more or less tendency to keratinization. The transformation of columnar epithelium into stratified squamous epithelium has been frequently noted in the bronchi. Such modified epithelium often has the appearance of invading the surrounding tissue, in a manner suggestive of the early stages of tumor growth.

Next in frequency to the primary tumors of the lung are a group of tumors classified as lymphomata. Of ten such tumors only two were externally apparent, four were primary in the thymus, one in the mesentery, and three were growing so diffusely that the exact site of their origin could not be determined. These tumors are composed of cells resembling more or less those of the lymphoid series, associated with a small amount of supporting tissue. The tumor tissue grows diffusely infiltrating normal tissues, and metastasis had occurred in all but one case. Secondary tumor nodules were found distributed throughout most of the body. One case in which tumor cells were distributed uniformly throughout the circulating blood is regarded as one of lymphatic leukemia. In several other cases the larger veins, especially those in close relation to tumor tissue, were filled with tumor cells, but it was thought that this condition might be a terminal one just preceding death.

Three tumors composed of cells of a primitive type are left unclassified. In these also the blood contained large numbers of tumor cells. These cells were not, however, evenly distributed throughout the circulation, but were more numerous in the veins. In one case clumps of tumor cells were adherent to the walls of the blood vessels.

In addition to tumors of the mammary gland such as have already been described by other authors, hypernephroma, papillary cystadenoma of the ovary, giant-cell sarcoma of the orbit, and sarcoma of the liver have been found in the mice kept under observation.

A larger number of primary tumors were found in mice which had never been inoculated than in those which had proved refractory to inoculable tumors. The majority of the tumors occurred in female mice, although several lymphomata and a considerable number of the lung tumors occurred in males.

Many of the primary tumors of the lung were associated with chronic inflammation and more or less marked modifications of the bronchial epithelium.

The frequency of tumors in three families of mice derived from three females with tumors indicates that the development of tumors is influenced by certain invisible inherited characters or qualities. A further analysis of data obtained from one of the females shows a greater frequency of tumors in offspring of parents one of which had a primary tumor than in offspring of parents neither of which had a tumor.

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DESCRIPTION OF PLATES.

PLATE XLIX.

FIG. 1. — Metaplasia of the epithelium of the common bile duct in the muskrat (Fiber zibethecus) due to the presence of parasitic worms.

FIG. 2. — A similar modification of the epithelium of the gall bladder of the same animal.

FIG. 3. — The same epithelium magnified to show its epidermoid character with differentiation into germinative, spiny, granule, and horny layers.

PLATE L.

FIG. 4. — The lung of a mouse showing a small bronchus containing crystals. The epithelium is somewhat modified in character in that it is no longer columnar, and is stratified in certain areas.

FIG. 5.— Papillary cystadenoma of the lung of a mouse. The tumor has extended into a small bronchus which is somewhat dilated. In such cases portions of the interior of the tumor are freely accessible to the inspired air.

FIG. 6.— Papillary cystadenoma of the lung of a mouse. Papillary processes of the tumor extend into a bronchus. The minute secondary tumor nodules seen in the upper left hand portion of the figure probably arose through the transplantation of tumor cells detached from papillary processes extending into the bronchi.

FIG. 7.— Primary epidermoid carcinoma of the lung of a mouse.

PLATE LI.

FIG. 8.— Lymphoma, primary in the thymus of a mouse. The indistinct whorls seen in the upper portion of the figure contain epithelial tissue, the remains of the entodermic anlage of the organ.

FIG. 9.— A metastasis of the tumor shown in Fig. 8, infiltrating the entire thickness of the myocardium. The endocardial surface is uppermost in the figure.

FIG. 10.— Lymphoma, primary in the mesentery of a mouse. Great variation in the size of the tumor cells occurred in both primary and metastatic tumor nodules. The smallest cells resemble the lymphocyte of the circulating blood, while the largest cells resemble the marrow giant cells, and there are cells of all intermediate sizes.

FIG. 11.— Liver infiltrated throughout with tumor tissue. The growth was of the nature of a lymphoma, and was primary in the mesentery. The cells are of uniform size and resemble large lymphocytes.

FIG. 12.— Lymphoma, primary in the thymus. Ciliated epithelium such as that described by Remak belonging to the involved thymus rather than to the tumor.

PLATE LII.

FIG. 13.— A generally disseminated lymphoma which infiltrated the lungs, liver, spleen, kidneys, and gastric mucosa. The blood vessels of the liver are to a large extent filled with tumor cells so that it resembles the liver in leukemia. This mouse belonged to Family B of the heredity experiments.

FIG. 14.— Lymphoma infiltrating the pancreas. Primary tumor situated in the mesentery. Same case as Figs. 11 and 15.

FIG. 15.— Lymphoma infiltrating the kidney and the tissue around the ureter. Same case as Figs. 11 and 14.

FIG. 16.— Liver infiltrated with tumor tissue which is found growing in the portal vein. (Case I. Tumor unclassified.) This tumor differs in character from the minute papillary cystadenoma of the lung in this case and is left unclassified.

PLATE LIII.

FIG. 17.— Same case as Fig. 16. The tumor has infiltrated the greater portion of the spleen and has grown into the splenic vein which it

occludes. The figure shows the attached border of the spleen with the splenic artery and occluded vein.

FIG. 18. — Another tumor infiltrating the liver. (Case II., unclassified.) The tumor cells are found in large numbers in the portal vein. The tumor cells also occurred in smaller numbers throughout the general circulation so that the case in many respects resembled a leukemia.

FIG. 19. — (Case III. Unclassified tumor.) Lymph node infiltrated with tumor which is composed of cells containing deeply stained cytoplasmic granules. This tumor was generally distributed throughout many of the organs and the point of origin could not be ascertained.

FIG. 20. — Liver showing portal vein occluded with tumor cells. Same case as Fig. 19.

FIG. 21. — A subcutaneous hemorrhagic tumor (Case V.) composed of cells without characteristic arrangement and showing no peculiarity by which they could be classified. The tumor cells are not concerned in the production of intercellular fibrils.

PLATE LIV.

FIG. 22. — Subcutaneous squamous-cell carcinoma (Case IV.) showing a high degree of keratinization. Certain portions of the tumor are glandular.

FIG. 23. — Hypernephroma (Case VII.). Kidney tissue in lower portion and tumor in upper portion of the figure.

FIG. 24. — Papillary cystadenoma of the ovary (Case VIII.). Irregular masses of epithelial tissue are found filling all available spaces.

FIG. 25. — Giant-cell sarcoma of the orbit of a mouse (Case XI.). Many of the tumor cells are associated with coarse fibroglia fibrils which are brought out with Mallory's phosphotungstic acid hematoxylin stain.

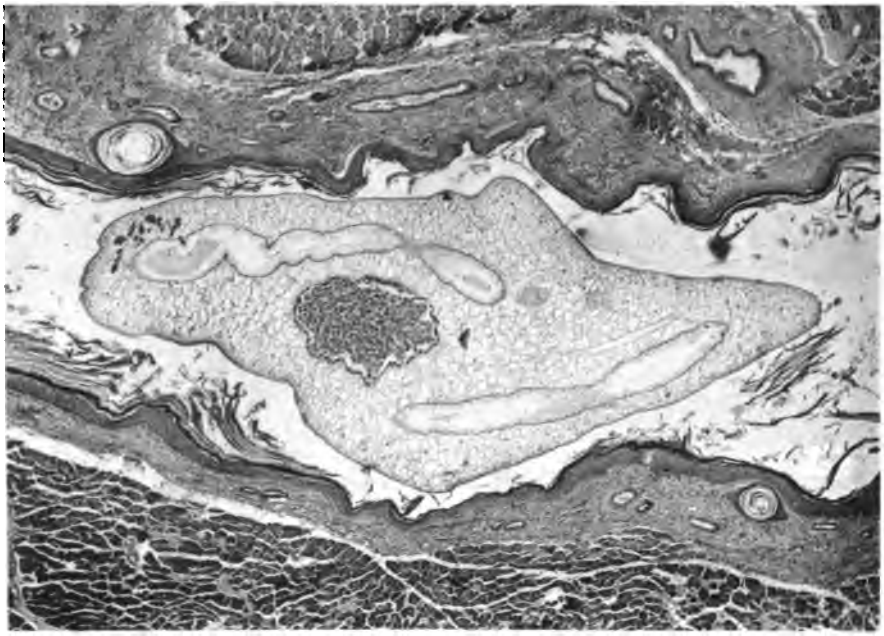
FIG. 26. — Liver of a mouse infiltrated with diffusely growing fibrosarcoma (Case XII.).

PLATE LV.

FIG. 27. — A tumor which developed spontaneously in a Japanese waltzing mouse. A portion showing a glandular arrangement of the epithelium.

FIGS. 28 and 29. — A tumor resulting from the first transplantation of the Japanese tumor shown in the preceding figure. Fig. 28 shows epithelium having a more or less glandular arrangement, and Fig. 29 taken from another portion of the same nodule shows only atypical connective tissue (sarcoma).

FIG. 30. — Alveolar arrangement of epithelium invariably found in the subsequent propagation of the Japanese tumor.



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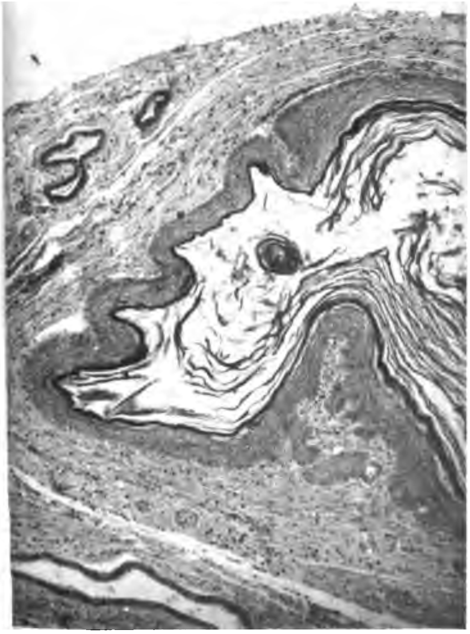

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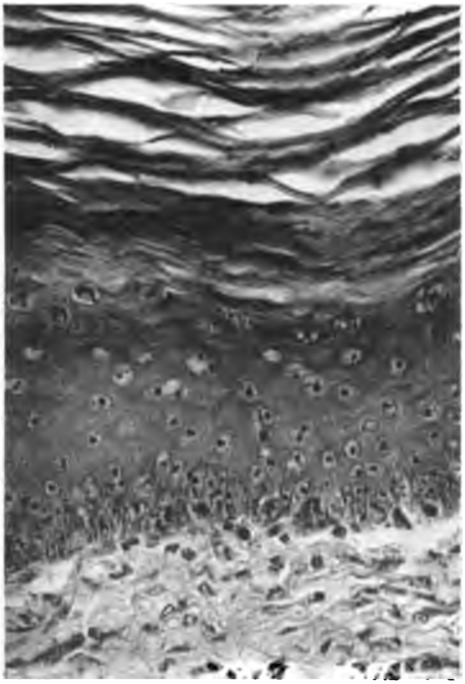

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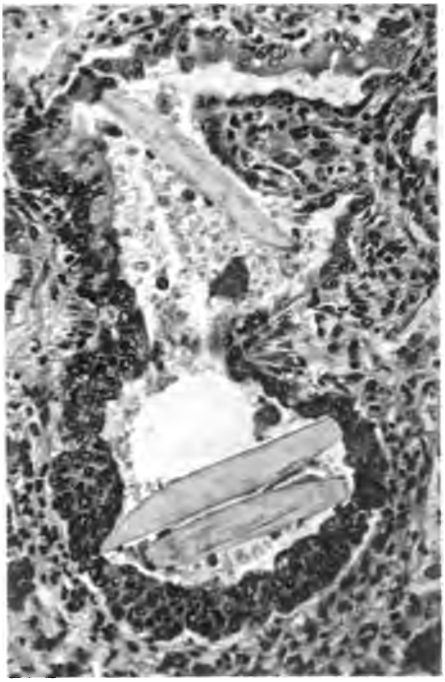

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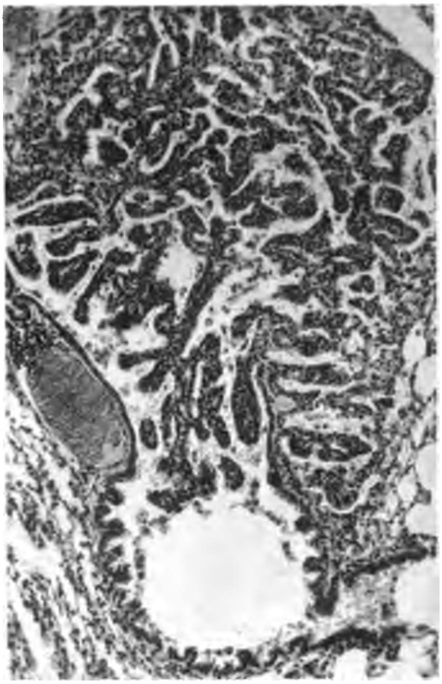

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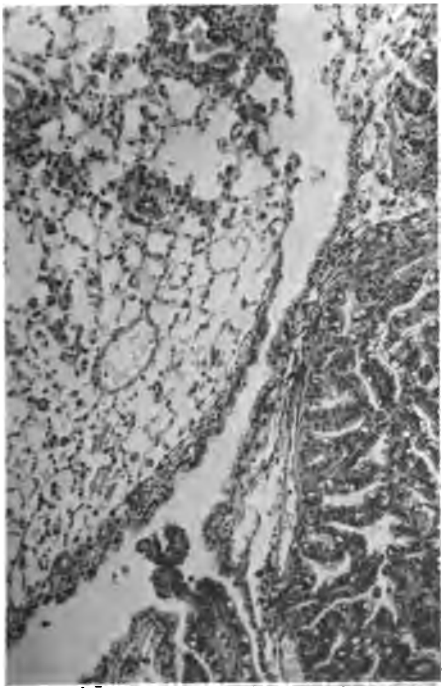

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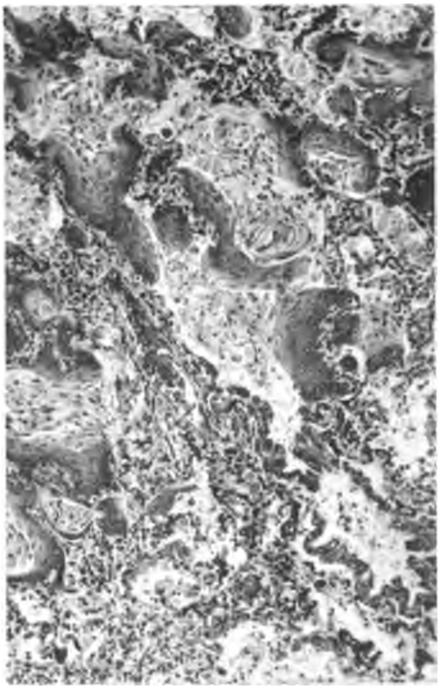

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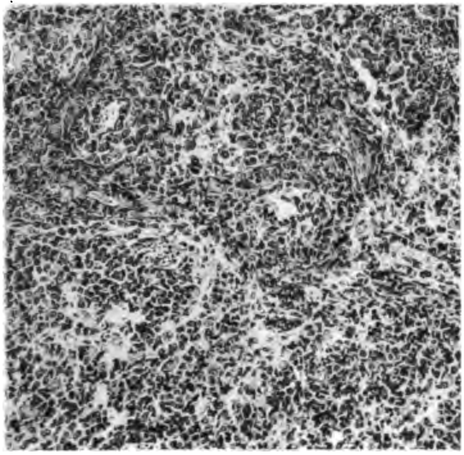

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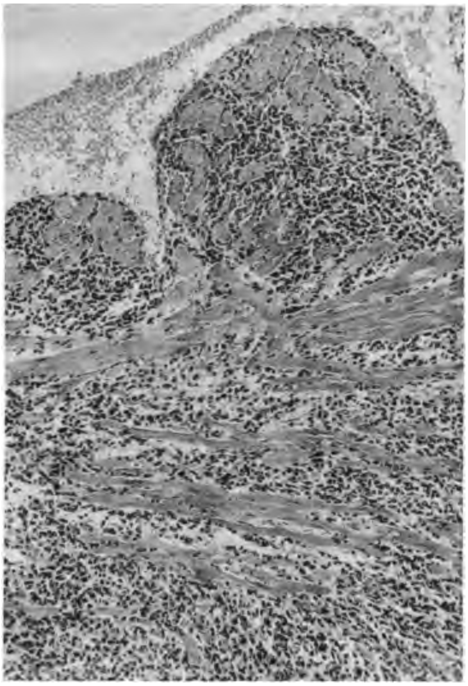

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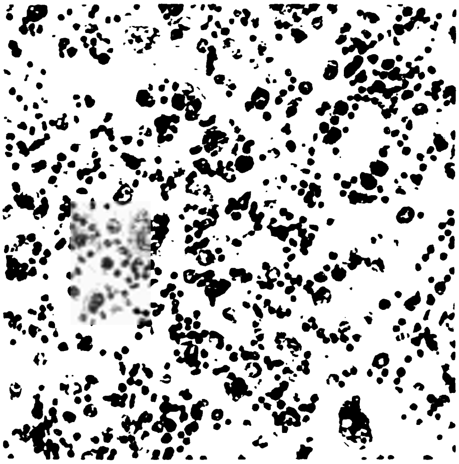

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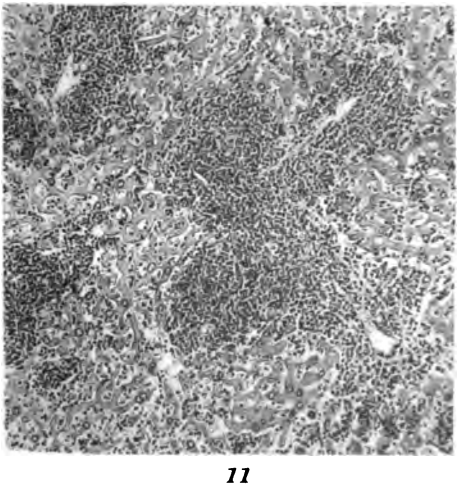

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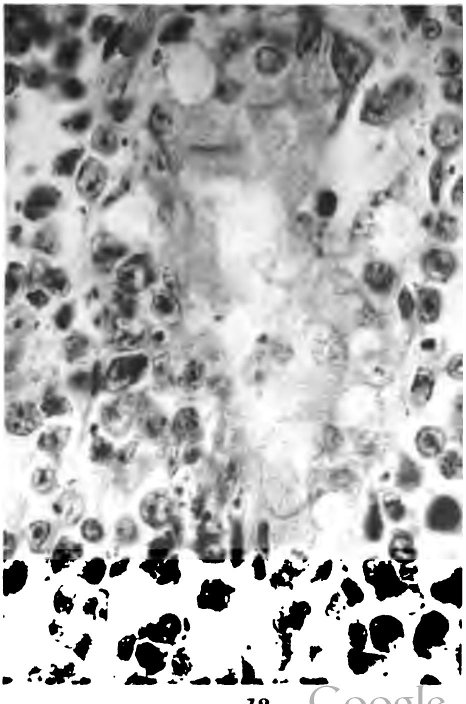

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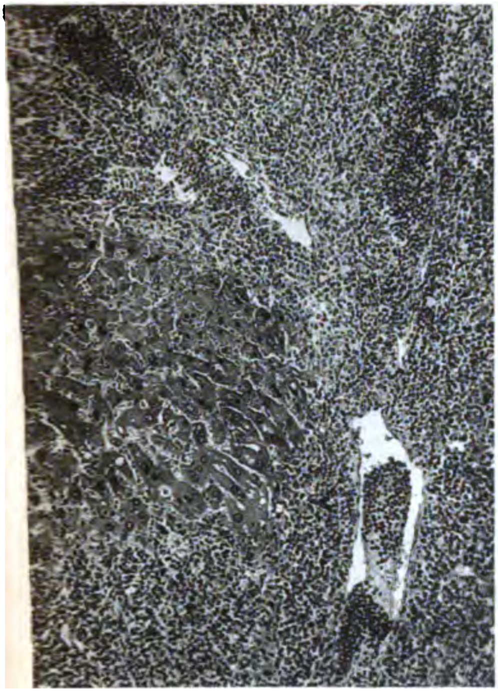

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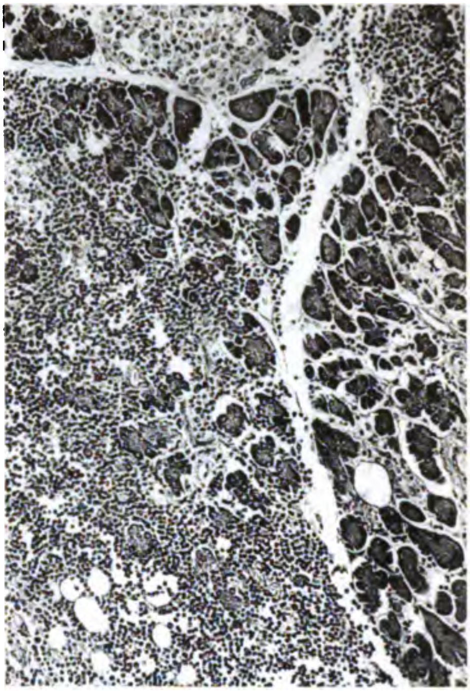

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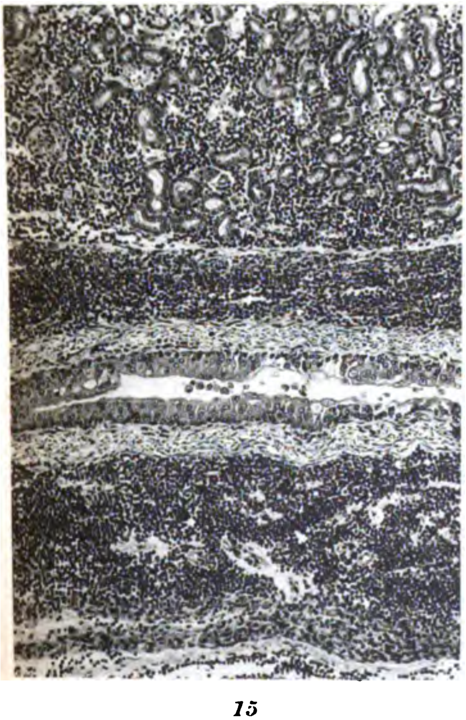

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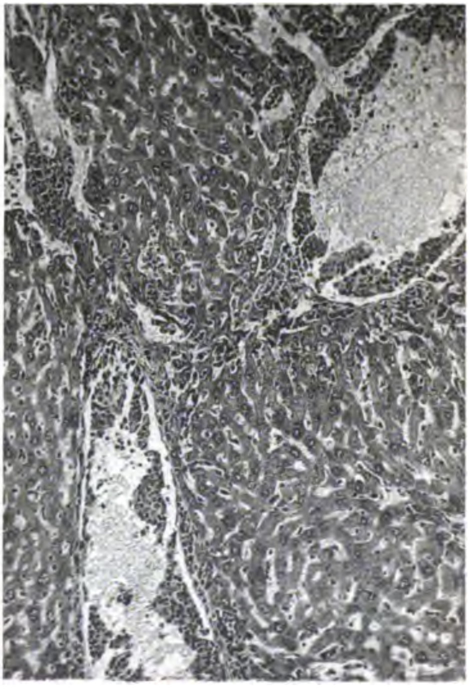

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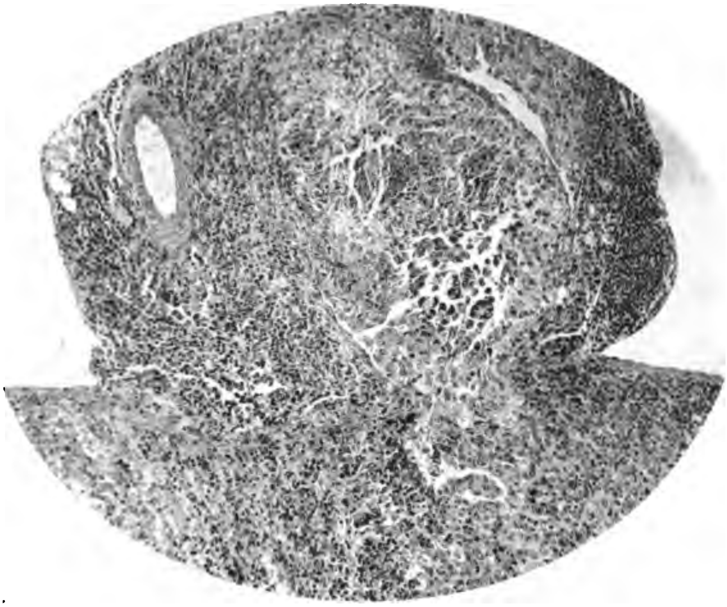

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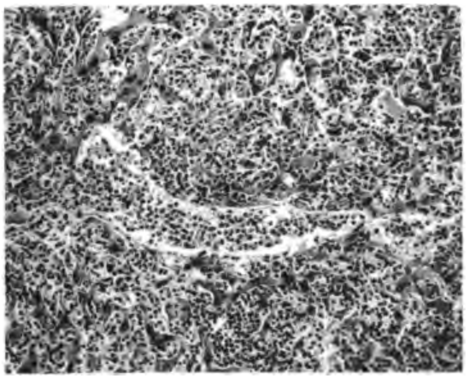

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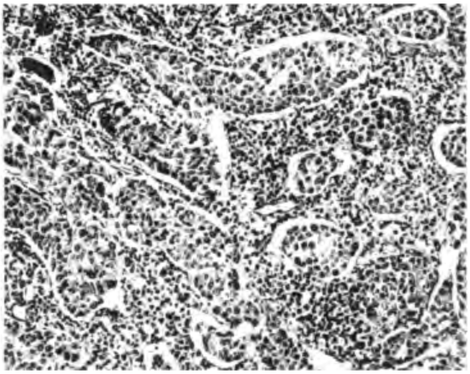

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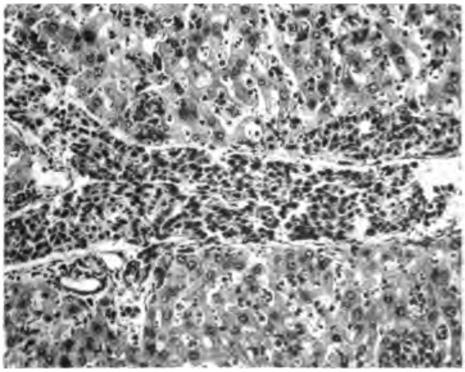

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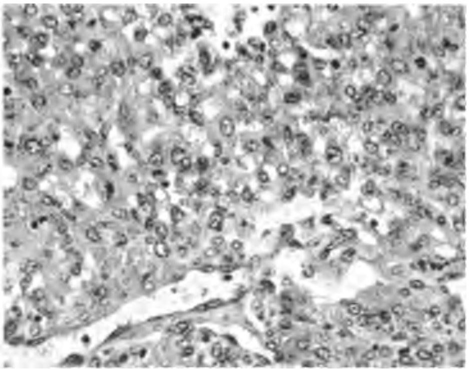

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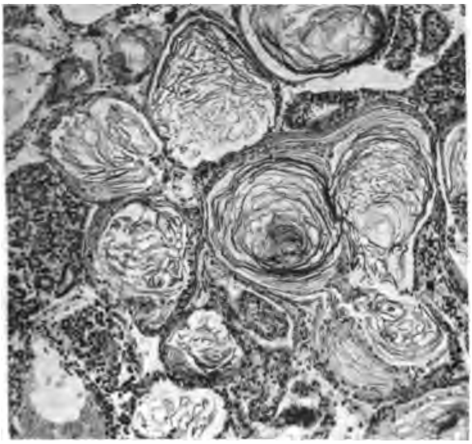

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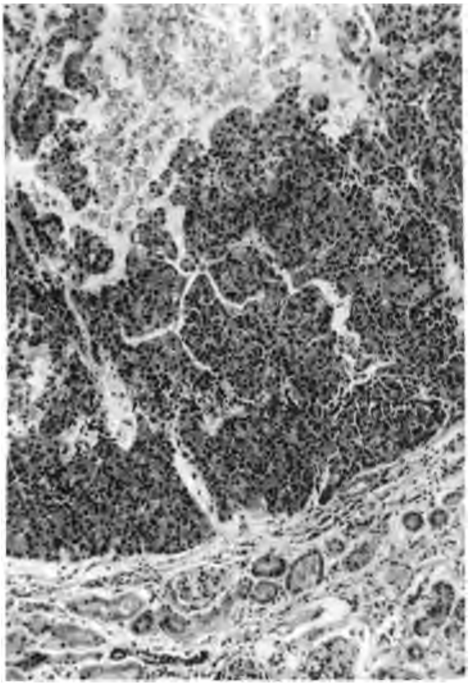

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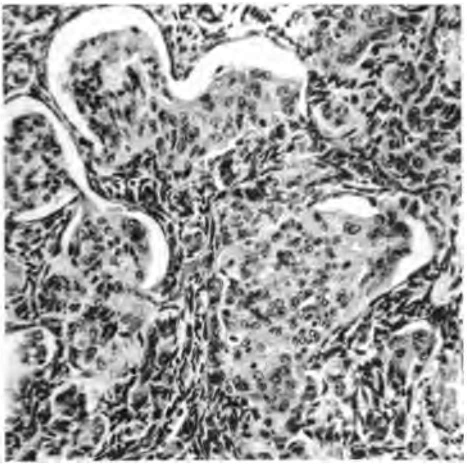

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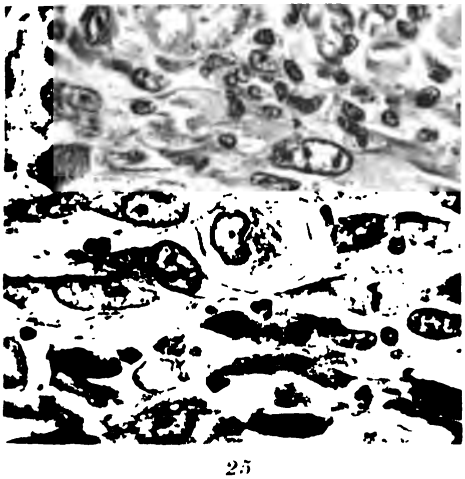

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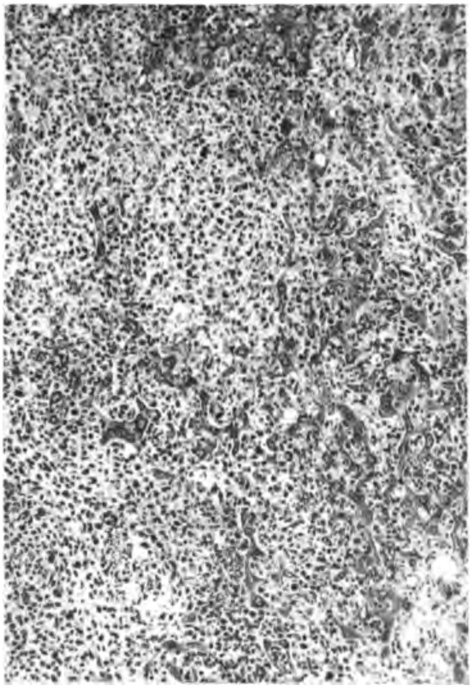

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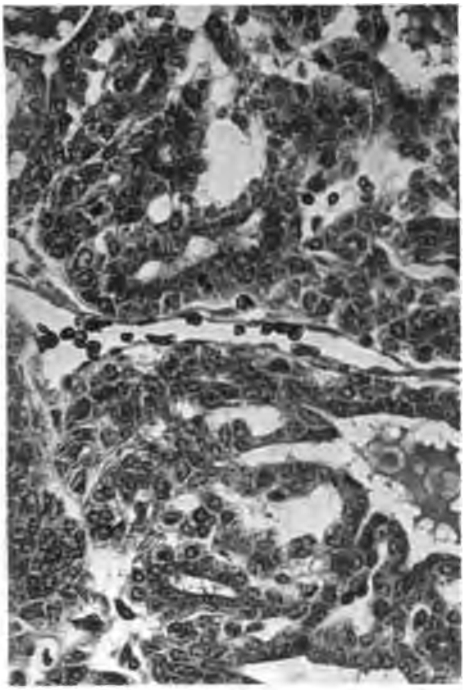

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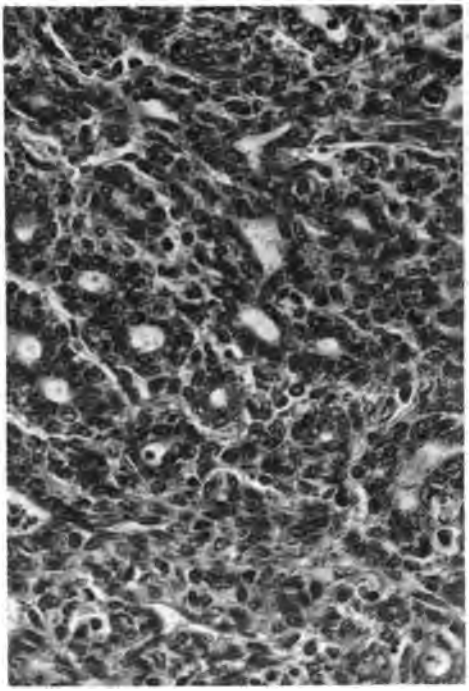

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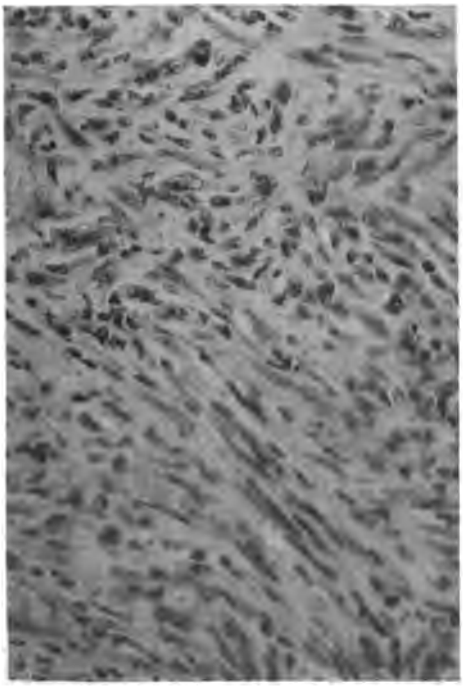

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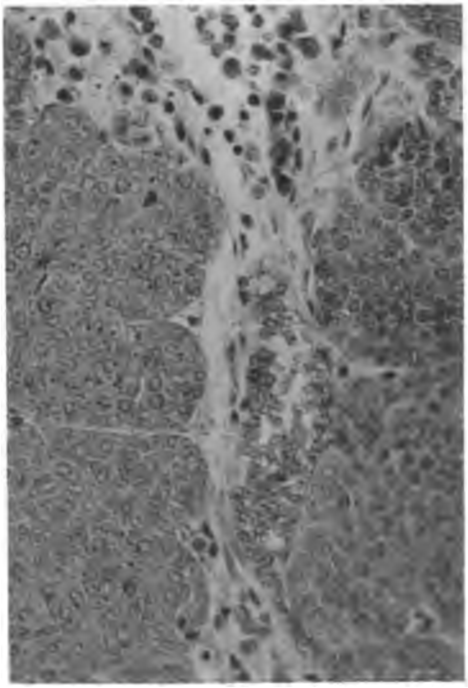

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A STUDY OF INHERITANCE IN MICE WITH REFERENCE TO THEIR SUSCEPTIBILITY TO TRANSPLANTABLE TUMORS.*

E. E. Tyzzer.

So far as can be ascertained, no systematic investigation has been made as to whether the susceptibility of mice to transplantable tumors is regularly transmitted from generation to generation, or whether it is inherited as a Mendelian character. It was the existence of a practically absolute difference in the susceptibility of common mice and Japanese waltzing mice to a tumor, which originated in a mouse of the latter variety, that led to the present study of the inheritance of this racial peculiarity.

Racial differences in the receptivity or susceptibility to transplantable tumors have been noted in mice by many investigators. Haaland[1] found that whereas a certain tumor grew on inoculation in nearly one hundred per cent of Berlin mice, it grew in only twenty-four per cent of Hamburg mice, and was practically innocuous to Christiania mice. He claims that susceptibility may vary also with change of location. Thus mice taken from Ehrlich's laboratory to Norway were found to be almost wholly insusceptible to a highly virulent sarcoma received directly from Ehrlich. He discusses the possible influence of change of climate and of variations of such conditions as heat, light, moisture, but concludes that diet is probably of greater importance. Jensen[2] has also observed distinct differences in the susceptibility of various stocks of rats to an inoculable sarcoma. A similar variation in the susceptibility of different stocks of rats to the Flexner-Jobling[3] tumor has recently been noted by Gay.[4] Loeb[5] found that a sarcoma which was readily propagated in white rats failed to grow when inoculated into colored and hybrid rats. He[6] also noted that a tumor which originated in a Japanese waltzing mouse grew on transplantation in nearly one hundred per cent of this variety of mice, but failed to

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* Received for publication July 26, 1909.

develop in common mice. The insusceptibility of certain breeds of mice to the Jensen tumor and of common mice to a transplantable tumor of Japanese waltzing mice was mentioned in the Fourth Report of this Commission.[7] Such results have been obtained so frequently that it is evident that the tissues of certain races of mice furnish a more favorable soil for the growth of implanted tumor cells than do the tissues of other races.

Various attempts have been made to correlate differences in susceptibility with certain anatomical peculiarities such as pigmentation of fur. Cuénot and Mercier[8] of the University of Nancy have recently investigated this question. They call attention to the occurrence in mice of numerous mutations which have been already defined by them. At least one hundred and twenty-eight varieties of mice, of which the following are a few examples, wild gray, white, black, yellow, waltzing or non-waltzing, pied or uniform in color, eyes black or red, etc., differing one from another by at least one determinant of the germ plasm, are recognized. Having at their disposal an almost complete collection of the possible varieties of mice, they have tested their susceptibility to a tumor which grew on inoculation in nearly one hundred per cent of Parisian white mice. As the result of its first inoculation into the Nancy race of mice, this tumor grew in only 16.6 per cent, but, as the result of later transplants, tumors developed in sixty-five and sixty-eight per cent of the animals. Thus a distinct racial difference between the mice of Paris and the mice of Nancy was apparent at the outset, although the tumor subsequently became adapted to the alien race, as was shown by the high percentage of successful results with later transplants. After a systematic inoculation of many different varieties of mice in their possession none were found to be either absolutely or relatively refractory to the inoculable tumor. There is, therefore, no correlation between susceptibility and certain recognized inherited characters, such as those determining the coloration of the fur, of the eyes, etc. These authors state that the difference in susceptibility between the mice of Paris and those of Nancy is, therefore, an invisible difference. The possible influence of diet is mentioned, but they think that the difference may be due to something of a more complicated nature.

The present investigation was undertaken in order to determine if the susceptibility to an inoculable tumor is transmitted in accordance with principles of heredity such as are embodied in Mendel's law. In the greater number of experiments an inoculable tumor which originally developed spontaneously in a Japanese waltzing mouse was used, although the Jensen and the Ehrlich Stamme II. tumors were also employed in certain instances in order to ascertain how generally applicable were the results obtained in experiments with the first named tumor. The waltzing mouse tumor was especially adapted for the study of the problem at hand on account of the uniformity of the results obtained from its inoculation into different varieties of mice. Thus from the first it grew in practically all Japanese waltzing mice inoculated, whereas repeated implantations in common tame mice were unsuccessful. The original tumor developed in a waltzing mouse which had been brought from Japan, but no difficulty was found in propagating it in Japanese waltzing mice reared in this country.

The histological structure of the tumor has already been described (see Fourth Report of the Cancer Commission of Harvard University, 1907, 49; Cf. Jour. Med. Research, 1907, XVII., 177). It resembles in most respects the tumor found by Loeb in a Japanese waltzing mouse. The epithelium in certain portions of the tumor is arranged in gland-like acini containing more or less colloid material; in other portions it is arranged in solid alveoli the central cells of which appear vacuolated from the presence of fatty material, so that there is considerable structural resemblance to the sebaceous and preputial glands; and a considerable part of the epithelium consists of flattened or spindle-shaped cells which are often intimately intermingled with connective tissue cells. The stroma is in places rather more cellular than is commonly found in spontaneous carcinomata of mice.

The first transplants of the tumor grew very slowly, a phenomenon which has been repeatedly observed in the artificial propagation of mouse tumors. Only three mice survived long enough for the tumor to attain any considerable development. In one of these a portion of the tumor nodule consisted largely of gland-like structures, whereas the remainder consisted of atypical connective tissue (illustrated in preceding paper). This tumor had attained a size only slightly larger than a pea in the nine months following its implantation. Unfortunately none of this tumor was used for inoculation, the strain having been propagated from the other more rapidly growing tumors of this generation. The portion of the nodule consisting of connective tissue was definitely limited from the surrounding tissue, and appeared to be of the nature of a new formation rather than of the character of a reaction to the tumor epithelium. It was of course impossible to determine at so long a period after the inoculation whether the sarcomatous portion of the nodule was derived from the host or from the implanted tumor. The appearance of sarcoma in this strain is of interest since Loeb several years previous to this had observed the development of sarcoma in a similar tumor which he succeeded in propagating in Japanese waltzing mice. Apart from this single case, all other tumors of this strain which have been thus far examined were epithelial in character. More or less of the gland-like arrangement of the epithelium, such as occurred in parts of the original tumor, persisted in the first two generations, and epithelial cells which were undergoing a fatty metamorphosis were found in considerable numbers. Neither of these features appeared, however, in subsequent generations, but the tumor maintained a constant type, its only important structural feature consisting of the arrangement of the epithelium in solid masses or alveoli with a relatively small amount of stroma.

The growth of this tumor even after long continued propagation is slow as compared with the Jensen tumor, and in several experiments the average weight of the tumors is only slightly in excess of .5 gram after five weeks' growth. If the tumor is allowed to continue its growth indefinitely, metastasis occurs in a large proportion of cases. Notwithstanding its slow growth this tumor has been found to be peculiarly adapted to the study of inheritance with reference to susceptibility. There is but slight tendency to necrosis, so that tumors of great size are often obtained in which there is little or no necrotic tissue. This makes possible a more uniform dosage of living tumor epithelium than can be obtained with tumors which are to a large extent necrotic. The inoculation results thus appear to be correspondingly more uniform than is the case with most of the transplantable tumors.

The characteristics of the Jensen and the Ehrlich St. II. tumors are well known. The Jensen tumor is a carcinoma the epithelium of which has an alveolar arrangement, whereas the Ehrlich tumor is an adenocarcinoma. Both originated in the subcutaneous tissue — presumably in the mammary gland — of the mouse.

In much of the investigation of the experimentally inoculable tumors there apparently has been too much dependence placed upon number and too little attention devoted to adequate control experiments, taking into account various conditions which may influence the nature of the results. The great difficulty lies in the impossibility of obtaining identical conditions in successive experiments. The influence of dosage on the result of the inoculation of tumor tissue has received considerable attention, and the age of the animals used has been taken into account by certain investigators.[14]

Apart from those instances in which marked racial differences have been met with, apparently but slight attention has been paid to the racial peculiarities of the animals employed in the propagation of tumors. When mice are obtained in large numbers from one or more dealers, variation in the nature of the stock used must be inevitable.

Variation in regard to susceptibility must always be taken into account in the determination of fluctuations in the growth of tumors. Thus, as I shall demonstrate later on, it is possible to produce by experimental breeding waltzing mice which are in every case insusceptible to a tumor that gives very nearly one hundred per cent of positive results in other waltzing mice. The alternation of such stocks would give fluctuations in infectivity from one hundred to no per cent.

In studying the differences in the susceptibility of various categories of mice it was necessary in each experiment to take into account certain known variable factors such as the dosage of tumor, the order of inoculation, and the age and general condition of the animals to be inoculated. In determining the influence of any one of these factors it is obviously impossible to make all associated conditions identical in the series to be compared. Recognizing, therefore, the inevitable variance of associated conditions, it is possible, however, either to make them approximately equivalent or to reverse the varying conditions in the course of successive experiments.

In all experiments the tumor tissue was implanted subcutaneously following the method employed by Bashford. The desired dose of living tumor was drawn into a trochar, which was inserted through the skin far back on the side of the animal and then thrust forward in the subcutaneous tissue toward the axilla, where its contents were deposited. The dose was estimated in each instance by the eye, so that there was necessarily more or less variation in the amounts received by different individuals. A dose considerably larger than that necessary to produce tumors in susceptible animals was inoculated in each case. The practice was followed of gently massaging the tissue about the site of inoculation in order to distribute the tumor tissue along the needle track so as to bring as much of it as possible into immediate relation with the tissues of the host. This was designed to increase the efficiency of the dose employed. In each experiment a single tumor was used to inoculate a comparative series of mice. Since it often required two to three hours to inoculate the total number of mice in a given experiment, there was a possibility of deterioration on the part of the tumor during this time. On this account the series of mice known to be susceptible were usually inoculated last, while the series of mice whose susceptibility was to be tested were inoculated first.

With the exception of a small number of mice of which only the approximate age was known, the exact age was recorded for each series. For comparative series, mice of approximately equal age were used as far as possible. Since it has become a matter of experience that the results are more uniform as well as more often positive in young animals, the supposedly insusceptible series was usually given the benefit of any difference of age. In certain experiments comparative series of old and young mice of the same variety were inoculated for the purpose of demonstrating the influence of age. Other conditions, such as general nutrition, etc., will be discussed later in connection with the individual experiments.

The results obtained in the following experiment illustrate a relative difference in the susceptibility of two races of common mice to two inoculable tumors. In this and in following experiments temporary tumors which disappear spontaneously are not recorded in the tables, although their occurrence is usually mentioned elsewhere. One of the two lots of mice used was purchased from a Providence dealer; the other lot was taken from a family of mice which has been bred for some time in the laboratory, and which originated from a mouse obtained from Buffalo, N.Y., mated with one obtained from Cambridge.

EXPERIMENT A.

Jan. 31, 1909. Ten Providence mice and ten mice of Buffalo-Cambridge stock were inoculated subcutaneously on the right side with the Ehrlich St. II. tumor.

Feb. 1, 1909. The same mice and also two other Providence mice and two other Buffalo-Cambridge mice were inoculated on the left side with the Jensen tumor.

The Providence mice were young adults not over two or three months old, while the Buffalo-Cambridge mice were all considerably older, the first five being six months and the remaining seven about four months old. All mice bearing tumors were killed on May 4, 1909. The results are tabulated below:

Variety of Mouse.	Results.		Remarks.
	Ehrlich Tumor.	Jensen Tumor.	
Providence No. 1	O	O	Died April 30.
" " 2	O	O	
" " 3	O	O	Tumors both wholly necrotic.
" " 4	O	O	
" " 5	+	O	Weight of tumor only .04.
" " 6	+	O	
" " 7	O	O	
" " 8	+	O	Jensen tumor necrotic.
" " 9	O	O	
" " 10	O	O	
" " 11	....	O	
" " 12	....	O	
Total positive	3 or 30%	O or O%	Tumors in 25% of entire series.
Buffalo-Cambridge No. 1	O	+	
" " " 2	O	+	
" " " 3	O	O	Nephritis.
" " " 4	O	O	
" " " 5	+	+	
" " " 6	+	+	
" " " 7	+	O	
" " " 8	+	+	
" " " 9	+	O	
" " " 10	O	O	
" " " 11	....	O	
" " " 12	....	O	
Total positive	5 or 50%	5 or 41⅔%	Tumors in 58⅓% of entire series.

Combined weight of tumors in Providence series...................... 13.845
Combined weight of tumors in Buffalo-Cambridge series 48.995

In addition to a difference in the degree of susceptibility shown by the two races of mice used in this experiment, a distinct difference in the infectivity is apparent in the two tumors used. The Jensen tumor failed to grow in the Providence mice, whereas the Ehrlich St. II. tumor grew in thirty per cent of those inoculated. In the other series there was an equal number of Jensen and Ehrlich tumors, and one or both developed in fifty-eight per cent of the mice.

The possibility of obtaining by selection a race of mice wholly immune to the inoculable tumors was considered. The mice which proved refractory in each experiment were used for breeding, and the offspring, together with control series of susceptible stocks of mice, have been inoculated from time to time. In Experiment B the Jensen tumor was inoculated three days previous to the Ehrlich tumor.

EXPERIMENT B.

July 3, 1908. Five offspring of the insusceptible Providence mice from Experiment A and five mice of mixed laboratory stock were inoculated on the left side with the Jensen tumor. The mice of both series were of equal age — sixty days.

July 6, 1908. The same mice were inoculated on the right side with the Ehrlich "Stamme II." tumor. The inoculations resulted as follows:

Variety.	Results.		Remarks.
	Ehrlich Tumor.	Jensen Tumor.	
Providence No. 1......	+	+	
" " 2......	O	O	
" " 3......	O	O	
" " 4......	O	O	
" " 5......	+	+	
Total positive.........	2	2	One or both tumors in 40%.
Laboratory No. 1......	O	+	Metastases in lungs.
" " 2......	+	+	
" " 3......	+	O	
" " 4......	O	+	
" " 5......	+	O	
Total positive..........	3	3	One or both tumors in 100%.

In this experiment the Providence race was shown to be less susceptible than a control series of mixed laboratory stock of equal age. An equal number of Jensen and of Ehrlich tumors developed.

EXPERIMENT C.

Jan. 26, 1909. Four offspring of the insusceptible Providence mice from Experiments A and B and four mice of Buffalo-Cambridge stock were inoculated with the Ehrlich St. II. tumor. Age of Providence mice, four months; of Buffalo-Cambridge mice, five months.

Variety.	Results.	Remarks.
Providence No. 1	0	
" " 2	0	
" " 3	0	
" " 4	0	Tumor sloughed off after long period of growth.
Total positive	0	Tumors in 0%.
Buffalo-Cambridge No. 1	+	
" " " 2	+	
" " " 3	+	
" " " 4	+	
Total positive	4	Tumors in 100%.

Only one tumor was inoculated in this instance. The great difference in the results with the two races of mice are apparent. The breeding of the refractory animals in the more insusceptible race will be continued with the end in view of obtaining a race which is absolutely rather than relatively insusceptible. The view held by certain investigators that inbreeding increases susceptibility to tumors is not borne out by the results of this and subsequent experiments.

The susceptibility of Japanese waltzing mice to the same two tumors of the common mouse was also tested.

Experiment D.

Five Japanese waltzing mice of unknown age and ten young adult common mice were inoculated on April 22, 1908, with the Jensen tumor and on the following day with the Ehrlich St. II. tumor No. 1925. The results are as follows:

Variety of Mouse.	Results.		Remarks.
	Ehrlich Tumor.	Jensen Tumor.	
Japanese waltzing No. 1.	— ?	0	Died 12 days after inoculation.
" " " 2.	0	0	Other four under observation 60 days or longer.
" " " 3.	0	0	
" " " 4.	0	0	
" " " 5.	0	0	
Total positive	0	0	Tumors in 0%.
Common No. 1	+	+	
" " 2	+	+	
" " 3	+	+	
" " 4	+	+	
" " 5	+	+	
" " 6	+	+	
" " 7	+	0	
" " 8	+	+	
" " 9	+	+	
" " 10	+	+	
Total positive	10	9	One or both tumors in 100%.

The ten common mice were killed at periods ranging from nineteen to fifty-one days after inoculation.

Combined weight of Ehrlich tumors in ten common mice	23.880
Combined weight of Jensen tumors in same	15.270
Total	39.150

In the four waltzing mice which survived neither the Jensen nor the Ehrlich tumor developed. Tumors developed in all the common mice, the Ehrlich tumor in ten, the Jensen tumor in nine. From this experiment it might appear that the Japanese waltzing mouse is not susceptible to these tumors, but since the animals used were of unknown age, but were apparently rather old, the evidence is not conclusive.

Further data concerning racial susceptibility to the same two inoculable tumors of the common mouse were obtained in Experiments E and F. The Jensen and the Ehrlich tumors were inoculated into small comparative series of common mice, Japanese waltzing mice, and hybrids* which had been obtained by cross-breeding these two varieties. Hybrids obtained from both Japanese waltzing and common mothers were used.

EXPERIMENT E.

April 25, 1908. Five Japanese waltzing, ten hybrids, and five common mice were inoculated subcutaneously with Jensen tumor No. 1917.

Variety of Mouse.	Age.	Result.	Remarks.
Common No. 1	1 month.	+	
" " 2	1 "	+	
" " 3	1 "	+	
" " 4	1 "	O	
" " 5	1 "	+	
Total positive	....	4	Tumors in 80%.
Hybrids C♂. J♀ No. 1 ...	2 months.	O	
" " " " 2 ...	2 "	O	
" " " " 3 ...	2 "	O	
" " " " 4 ...	2 "	O	
" " " " 5 ...	2 "	O	
Total positive	....	O	Tumors in O%.

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* A full description of these hybrid mice will be given further on in this paper.

Experiment E. — *Continued.*

Variety of Mouse.	Age.	Result.	Remarks.
Hybrids J♂. C♀ No. 1 ...	1½ months.	0	
" " " " 2 ...	1½ "	0	
" " " " 3 ...	1½ "	0	
" " " " 4 ...	1½ "	0	
" " " " 5 ...	1½ "	0	
Total positive	....	0	Tumors in 0%.
Japanese waltzing No. 1 ...	2 months.	Dead 7	days after inoculation.
" " " 2 ...	2 "	" 19	" " "
" " " 3 ...	2 "	0	
" " " 4 ...	2 "	0	
" " " 5 ...	2 "	0	
Total positive	....	0	Tumors in 0%.

The Jensen tumor grew to large size in four (eighty per cent) of the five common mice, but failed to develop in both sorts of hybrids and in the waltzing mice which survived. The tumor used in this experiment was not considered especially favorable for inoculation, but it nevertheless grew in four of the five common mice. The tumors developed for a time in many of the hybrids and appeared as well-defined nodules ten days after the inoculation, but were, with one exception, absorbed during the following week. This temporary growth took place in two hybrids of a Japanese waltzing mother, and in all five hybrids of a common mother. A second inoculation of the three surviving waltzing mice and the ten hybrids, thirty-four days later, with the Jensen tumor resulted negatively in the waltzing mice, and in one tumor which grew to large size in one of the hybrids. Of the nine hybrids, in which the reinoculation of the Jensen tumor resulted negatively, seven were in a subsequent experiment inoculated with the Japanese waltzing mouse tumor with the result that this tumor developed in all.

The susceptibility of these varieties of mice to the Ehrlich tumor was also determined.

Experiment F.

April 25, 1908. Five Japanese waltzing mice, ten hybrids, and five common mice were inoculated subcutaneously with the Ehrlich St. II. tumor No. 1923.

Variety of Mouse.	Age.	Weight of Tumor after				Remarks.
		33 days.	37 days.	48 days.	65 days.	
Common No. 1	2 mos.	4.170				
" " 2	2 "	1.870				
" " 3	2 "	3.140				
" " 4	2 "	4.730				
" " 5	2 "	2.290				
Total weight	....	16.200	....	....	....	Tumors in 100%.
Hybrids C♂. J♀. No. 1	1 mo.	0	0	0	0	
" " " " 2	1 "	3.270				
" " " " 3	1 "	Small.	......	....	2.800	
" " " " 4	1 "	0	0	0	0	
" " " " 5	1½ "	0	0	0	0	
Total weight	....	3.270+	....	....	2.800	Tumors in 40%.
Hybrids J♂. C♀. No. 1	1½ mo.	2.905				
" " " " 2	1½ "	Small.	....	....	6.450	
" " " " 3	1½ "	0	0	0	0	
" " " " 4	1½ "	0	0	0	0	
" " " " 5	1½ "	2.200				
Total weight	....	5.105+	......	....	6.450	Tumors in 60%.
Japanese waltzing No. 1	1 mo.	Died 11 days after inoculation.				Tumor living.
" " " 2	1 "	Small.	....	2.680		
" " " 3	1 "	Small.	....	....	.950	
" " " 4	1 "	Small.	.330			
" " " 5	1 "	0	0	0	0	
Total weight	....	....	.330+	2.680+	.950	Tumors in 75%.

The five hybrids in which the Ehrlich tumor failed to grow were reinoculated thirty-three days later with the same tumor with the result that it failed to develop in four, and grew in one, which was inoculated at the same time with the Japanese waltzing mouse tumor.

Of the five common mice inoculated all (one hundred per cent) developed tumors. Tumors were present in all five hybrids of a Japanese mother at the end of a fortnight, but in three the tumors later disappeared, while in two (forty per cent) they developed to great size. Tumors likewise developed in all hybrids of a common mother: two disappeared spontaneously, two grew rapidly, and one remained stationary for a time, but later grew rapidly. Positive results were thus obtained in sixty per cent. Of the five Japanese waltzing mice, one died eleven days after inoculation and presented a small mass of living tumor. Three (seventy-five per cent) of the remaining four developed tumors which, however, varied greatly in size and were all much more slowly growing than the tumors in the control series of common mice.

From the data furnished by these experiments a number of conclusions may be drawn. Certain races of common mice show relative differences in susceptibility to the Jensen and to the Ehrlich tumors. The differences are maintained through successive generations, even though all the respective races are kept under essentially identical conditions, *i.e.*, in the same room and fed with the same food. The Ehrlich tumor is capable of growing in varieties of mice in which the Jensen tumor invariably fails. Thus the Jensen tumor failed to grow in both old and young waltzing mice, whereas the Ehrlich tumor grew in young mice of this variety. Japanese waltzing mice are thereby less susceptible than common mice to the inoculable tumors of the latter. Young hybrids of both common and Japanese waltzing mothers are in a certain number of cases susceptible to the Ehrlich tumor and in fewer cases susceptible to the Jensen tumor. These tumors attained slightly greater development in hybrids of a common mother than in hybrids of a Japanese waltzing mother, although the numbers used were too small to attach great importance to this point. With respect to the number of tumors which developed, young hybrids, however, appeared to be no more susceptible to the Ehrlich tumor than waltzing mice of similar age. Since in all these experiments the difference in susceptibility of the various races of mice to the Jensen and the Ehrlich tumors are for the most part relative rather than absolute, it did not appear that these tumors were especially favorable for the study of the inheritance of susceptibility. The variability in the biological behavior of these tumors had to be always taken into consideration. More extensive experiments were planned with reference to susceptibility to the Japanese waltzing mouse tumor, which appeared to be especially adapted for such investigation.

Since this tumor has in every instance failed to develop when inoculated into common mice, the cross-breeding of Japanese waltzing and common mice was undertaken with the end in view of testing the susceptibility of the hybrids thus produced. With the exception of a few lots of animals which are in each case designated, all hybrids used in the two preceding and all subsequent experiments were derived from the reciprocal cross-breeding of four common albino mice, two of each sex, with four black and white Japanese waltzing mice, two of each sex. The four Japanese mice were presumed to be susceptible to the tumor, since up to that time it had developed in one hundred per cent of all such mice inoculated. The actual susceptibility of these four mice could not be tested, for the obvious reason that the development of the tumor would make the necessary amount of breeding from them impossible. The four common white mice were inoculated with negative result after a sufficient number of hybrids had been obtained. On account of their age at the time of this inoculation, the result of the inoculation is of less importance than the fact that the tumor has in every instance failed to grow in young mice of the same stock.

The offspring derived from the cross-breeding of common albino mice and black and white Japanese waltzing mice present features of considerable interest, in addition to certain recognized inherited characters such as the coat coloration, the waltzing habit, etc. Since most of the points have been covered in the investigations of Haacke,[9] von Guaita,[10] Cuénot,[11] and others, such data will be but briefly considered here.

No distinctive difference was noted in the hybrids (F_1) derived from the mating of common males with Japanese waltzing females as compared with those derived from Japanese waltzing males and common females. The mice of this first filial generation (F_1) were invariably lively, vigorous mice, of rather wild or excitable disposition. They were considerably larger than the waltzing mice and somewhat smaller than the common mice from which they were derived. In none was there any tendency to waltz, so that the waltzing habit is to be considered a recessive character. With frequent handling much of the "wildness" of disposition disappeared, although it persisted in certain individuals who remained excitable and vicious when mature. In the mice of this generation either the entire coat or the greater part of it was pigmented. In those in which the pigmentation was incomplete there was a white streak on the forehead and on the belly. Thus, as has been noted by other investigators, the pigmented area is much greater in the hybrids than in the partially pigmented or spotted parent. Hybrids of the following coloration were derived from the several matings: gray, gray with white streak on forehead and belly, and black. One of the common females on being subsequently mated with a different Japanese male gave birth to two spotted black and white, two black, one gray, and three gray with white streak on forehead and belly, in a single litter of young.

In the second generation (F_2) differences of temperament were noted. In a single litter certain individuals were of gentle disposition, whereas others were wild and vicious. The waltzing character appeared again in this generation, but the number of mice which waltzed fell somewhat short of the Mendelian expectation. In the fifty-four mice of this generation inoculated, ten or 18.5 per cent waltzed, but waltzing mice were known to have previously died from several of the litters taken for these experiments. This generation presented greater variation in color and in color distribution than the preceding. In addition to gray, black, and these colors combined with narrow streaks of white, there were spotted mice with a greater amount of white, pure albinos, and spotted brown and white with brown eyes. The color and the waltzing characters were inherited independently of one another, just as has been found concerning the inheritance of other Mendelian characters. Thus in this generation there were gray, gray and white, black, black and white, brown, and albino mice which waltzed. They also differed in other respects than in color from the pure bred Japanese waltzing mouse. The size attained by certain individuals greatly exceeded that of the latter variety, and approximated that of the common mouse. The wild disposition was often combined with the waltzing habit, so that when disturbed peculiar movements were performed, which were never observed in the true Japanese waltzing mouse. They often rolled over or turned somersaults in an effort to escape. Coördination developed late in many of the young which turned out to be waltzers. These, even when more than half grown propelled themselves by irregular convulsive movements, and, after they had learned to use their feet, they frequently fell or rolled completely over.

Although such waltzing mice may breed true with regard to the waltzing habit, they, nevertheless, differ essentially from the so-called Japanese waltzing variety from which they have originated. Thus purity may be attained with respect to a single character such as waltzing, but with the recognition of numerous other characters the chance of the reoccurrence of the combination of characters peculiar to a given variety or breed becomes progressively smaller.

In breeding for the third generation (F_3) the mating of the animals was purposely made promiscuous rather than selective. In the sixteen mice of this generation used for inoculation only one waltzed.

In the following experiments the susceptibility of the various series of mice to the inoculable tumor of the Japanese waltzing mouse was tested. The mice are numbered in the order of their inoculation, and are arranged in comparative series so that certain series of mice serve as controls to others, the susceptibility of which is to be tested. The object of the first experiment was to determine if the hybrids (F) obtained by mating a common male with a Japanese waltzing female are susceptible. Since at this time the tumors were of small size, two tumors were required to inoculate the total number of mice. Series of hybrids, series of common mice, and series of Japanese waltzing mice were inoculated at the same time with the two tumors.

EXPERIMENT I.

March 17, 1908. Five common mice, five hybrids, and five Japanese waltzing mice, Nos. 1938 to 1952 inclusive, were inoculated subcutaneously in the region of the right axilla with tumor No. 1873, which had been growing seventy-seven days in a Japanese waltzing mouse.

March 18, 1908. Three similar series of mice, Nos. 1953 to 1968 inclusive, were inoculated in the same manner with tumor No. 1886, which had grown sixty-three days in a Japanese waltzing mouse.

Number.	Variety of Mouse.	Age.	Death.	Weight of Tumor.
1938.....	Common.	45 days.	43 days after inoc.	0
1939.....	"	45 "	24 " " "	0
1940.....	"	80 "	Alive 3½ mos. " "	0
1941.....	"	80 "	" 3½ " " "	0
1942.....	"	80 "	" 3½ " " "	0
1943.....	Hybrid F (C♂.J♀).	5½ weeks.	77 days after inoc.	2.380
1944.....	" " "	5½ "	49 " " "	3.855
1945.....	" " "	5½ "	77 " " "	1.320
1946.....	" " "	5½ "	77 " " "	9.820
1947.....	" " "	5½ "	32 " " "	.977
1948.....	Japanese waltzing.	2 months.	41 days after inoc.	.134
1949.....	" "	2 "	78 " " "	.115
1950.....	" "	2 "	78 " " "	.930
1951.....	" "	2 "	55 " " "	.480
1952.....	" "	2 "	81 " " "	1.720
1953.....	Japanese waltzing.	2 months.	80 days after inoc.	2.900
1954.....	" "	2 "	77 " " "	.330
1955.....	" "	2 "	77 " " "	2.180
1956.....	" "	2 "	76 " " "	4.450
1957.....	" "	2 "	77 " " "	.160

EXPERIMENT I. — *Continued.*

Number.	Variety of Mouse.	Age.	Death.	Weight of Tumor.
1958.....	Hybrid F (C♂. J♀).	5½ weeks.	71 days after inoc.	3.750
1959.....	" " "	5½ "	76 " " "	2.150
1960.....	" " "	5½ "	80 " " "	3.840
1961.....	" " "	5½ "	49 " " "	1.570
1962.....	" " "	5½ "	80 " " "	1.370
1963.....	" " "	5½ "	76 " " "	1.230
1964.....	Common.	81 days.	Alive 10 weeks after inoc.	0
1965.....	"	81 "	" 10 " " "	0
1966.....	"	81 "	" 10 " " "	0
1967.....	"	81 "	" 10 " " "	0
1968.....	"	81 "	" 10 " " "	0

All of the eleven hybrids developed tumors as well as the control series of ten Japanese waltzing mice. The ten common mice inoculated at the same time, with the same material, developed no tumors. The tumors grew on the average more rapidly in the hybrids than in the Japanese waltzing mice. The hybrids were, however, younger than the Japanese waltzing mice. Subsequent experiments were planned so as to yield data concerning the influence of age, size, and body growth on the rate of tumor growth.

Having established the susceptibility of the hybrid offspring of common male and Japanese waltzing female mice, the next point to be determined was whether the hybrid offspring of Japanese waltzing male and common female mice are susceptible. A single tumor was inoculated into forty-seven mice arranged in comparative series. Ten mice of each of the series in which tumors developed were killed thirty-six days after the inoculation, and the tumors weighed in order to estimate the comparative rates of growth.

EXPERIMENT 2.

June 2, 1908. Tumor No. 1956, which growing in a Japanese waltzing mouse had attained the weight of 4.450 grams in seventy-six days, was inoculated into the following comparative series of first generation hybrids, Japanese waltzing mice, and common mice in the order of their enumeration.

Number.	Variety of Mouse.	Age.	Death.	Weight of Tumor.
2073.....	Hybrid F_1 (J♂. C♀)	5 weeks.	36 days after inoc.	1.850
2074.....	" " "	5 "	36 " " "	5.195
2075.....	" " "	5 "	36 " " "	3.315
2076.....	" " "	5 "	36 " " "	4.950
2077.....	" " "	5 "	36 " " "	2.790
2078.....	" " "	5 "	36 " " "	.300
2079.....	" " "	5 "	36 " " "	3.590
2080.....	" " "	5 "	36 " " "	2.060
2081.....	" " "	5 "	36 " " "	2.940
2082.....	" " "	12 "	36 " " "	1.045
Total..	10 Hybrids F_1 (J♂.C♀)	....	....	28.035
2084.....	Hybrid F_1 (C♂. J♀)	7 weeks.	36 days after inoc.	2.530
2085.....	" " "	7 "	36 " " "	1.120
2086. ...	" " "	7 "	36 " " "	1.160
2087.....	" " "	7 "	36 " " "	.390
2088.....	" " "	7 "	36 " " "	2.750
2089.....	" " "	7 "	36 " " "	2.300
2090.....	" " "	7 "	36 " " "	1.080
2091.....	" " "	7 "	36 " " "	2.910
2092.. ..	" " "	7 "	36 " " "	1.260
2093.....	" " "	7 "	36 " " "	.890
Total..	10 Hybrids F_1 (C♂. J♀)	....	....	16.390
2094.....	Common.	6 weeks.	18 days after inoc.	0
2095.....	"	6 "	Alive 2½ mos. " "	0
2096.....	"	6 "	" " " " "	0
2097.....	"	6 "	28 days " "	0
2098.....	"	6 "	18 " " "	0
2099.....	"	6 "	Alive 2½ mos. " "	0
2100.....	"	6 "	" 2½ " " "	0
2101.....	"	6 "	" 2½ " " "	0
2102.....	"	43 days.	" 2½ " " "	0
2103.....	"	43 "	" 2½ " " "	0
Total..	10 Common.	....	....	0

EXPERIMENT 2. — *Continued.*

Number.	Variety of Mouse.	Age.	Death.	Weight of Tumor.
2104.....	Japanese waltzing.	5 weeks.	36 days after inoc.	.995
2105.....	" "	5 "	36 " " "	1.670
2106.....	" "	5 "	36 " " "	1.850
2107.. ..	" "	5 "	36 " " "	1.450
2108.. ..	" "	5 "	36 " " "	.900
2109.....	" "	5 "	36 " " "	2.550
2110.....	" "	5 "	36 " " "	1.240
2111.....	" "	5 "	36 " " "	.475
2112.....	" "	5 "	36 " " "	.720
2113.....	" "	5 "	36 " " "	.370
Total..	10 Japanese waltzing.	....	....	12.220
2114.....	Japanese waltzing.	5 weeks.	84 days after inoc.	4.670
2115.....	" "	5 "	21 " " "	1.050
2116.....	Young common.	12 days.	Alive 2½ mos. " "	0
2117....	" "	12 "	" 2½ " " "	0
2043.....	Hybrids F_1 (J♂. C♀)	12 weeks.	27 days " "	.580
2083.....	" " "	12 "	91 " " "	11.110

Tumors developed in all hybrids derived from common females as well as in all hybrids derived from Japanese waltzing females. In a comparative series of ten common mice no tumors developed. The combined weight of the tumors in ten hybrids (J ♂ × C ♀) more than doubles that of the tumors in ten Japanese waltzing mice, although the two series are of the same age. The tumors also grew more rapidly in hybrids (C ♂ × J ♀) than in Japanese waltzing mice. The fact that the latter were the last to be inoculated may have influenced the results to some extent. The fact that both categories of hybrids proved susceptible to the tumor indicated that the susceptibility is transmitted by the male as well as by the female.

Since it was thought possible that the tumor might grow in very young common mice, this occasion was taken to test their susceptibility. Two young nursing mice, Nos. 2116 and 2117, aged twelve days, were inoculated, but the tumor failed to grow in these as well as in older mice. Hybrid No. 2043, which had been inoculated with the Ehrlich tumor, was inoculated also with the Japanese tumor with the result that both tumors grew.

Further experiments were planned to determine if susceptibility to the tumor is transmitted in accordance with Mendel's law of heredity. On this hypothesis it would be necessary to consider susceptibility as a dominant character, since it is demonstrable in all the hybrid offspring. The hybrids of the first generation (F_1) were bred together, and the susceptibility of the second generation (F_2) was tested.

EXPERIMENT 3.

June 29, 1908. Tumor No. 2115, which had attained the weight of 1.050 grams twenty-one days after its implantation in a Japanese waltzing mouse, was inoculated into six hybrids of the second generation (F_2), seven Japanese waltzing mice, and seven common mice.

Number.	Variety of Mouse.	Age.	Death.	Weight of Tumor.
2125	Hybrid F_2.	7 weeks.	Alive 2½ mos. after inoc.	0
2126	" "	7 "	" 2½ " " "	0
2127	" "	7 "	" 2½ " " "	0
2128	" "	7 "	" 2½ " " "	0
2129	Japanese waltzing.	10 weeks.	36 days after inoc.	.430
2130	" "	10 "	36 " " "	.340
2131	" "	10 "	36 " " "	.290
2132	" "	10 "	36 " " "	.320
2133	" "	10 "	36 " " "	.520
2134	" "	10 "	36 " " "	.560
2135	" "	10 "	24 " " "	Not weighed.
2136	Hybrid F_2.	31 days.	Alive 2½ mos. after inoc.	0
2137	" "	31 "	" 2½ " " "	0
2138	Common.	29 days.	Alive 2½ mos. after inoc.	0
2139	"	29 "	" 2½ " " "	0
2140	"	29 "	" 2½ " " "	0
2141	"	29 "	" 2½ " " "	0
2142	"	29 "	" 2½ " " "	0
2143	"	29 "	" 2½ " " "	0
2144	"	29 "	" 2½ " " "	0

Of the series of six mice of the second generation (F_2) tumors developed in none. Tumors developed in all of a control series of seven Japanese waltzing mice, and in none of a series of seven common mice. In order to test in a thorough manner the susceptibility of generation F_2, further experiments of a similar nature were necessary to obtain the sufficient number of results.

EXPERIMENT 4.

July 23, 1908. Five hybrids of the second generation (F_2), five Japanese waltzing, and five hybrids of the first generation (F_1) were inoculated by the usual method with tumor No. 2135, which had grown twenty-four days in a Japanese waltzing mouse.

Number.	Variety of Mouse.	Age.	Death.	Weight of Tumor.
2162	Hybrid F_2.	45 days.	Alive 4 mos. after inoc.	0
2163	" "	45 "	" " " "	0
2164	" "	45 "	" " " "	0
2165	" "	45 "	" " " "	0
2166	" "	45 "	" " " "	0
Total ...	5 Hybrids F_2.	....	....	0
2167	Japanese waltzing.	45 days.	48 days after inoc.	.650
2168	" "	45 "	48 " " "	.420
2169	" "	45 "	48 " " "	.720
2170	" "	45 "	48 " " "	.590
2171	" "	45 "	48 " " "	.550
Total ...	5 Japanese waltzing.	....	....	2.930
2172	Hybrid F_1.	14 weeks.	48 days after inoc.	.360
2173	" "	14 "	48 " " "	.570
2174	" "	14 "	48 " " "	1.690
2175	" "	18 "	48 " " "	.470
2176	" "	18 "	48 " " "	.350
Total ...	5 Hybrids F_1.	....	....	3.440

No tumors developed in five mice of generation F_2, whereas tumors developed in all mice of the control series of Japanese waltzing mice and hybrids of generation F_1. The ages of generation F_2 and of the Japanese waltzing mice were in this instance identical. The mice of generation F_1 were considerably older.

In the following experiment it was necessary to employ three tumors in the inoculation of the thirty mice whose susceptibility was to be tested. Comparative series were in each instance inoculated with a single tumor. In order to determine the influence of age on the rate of tumor growth, series of young and old Japanese waltzing mice were inoculated. The susceptibility of the four common parents (P_1), and of generation F_1 hybrids, obtained by crossing Japanese waltzing mice with common mice of another stock, was tested. (The latter are indicated as "alien" in the table, Experiment 5.)

EXPERIMENT 5.

Aug. 4, 1908. Three tumors, Nos. 2129, 2132, 2133, which had been growing thirty-six days in Japanese waltzing mice and which weighed .430, .320, and .520 respectively, were used to inoculate comparative series of second generation (F_2) hybrids, Japanese waltzing mice, first generation (F_1) hybrids including four derived from an alien stock, and four common mice, the parents of the hybrids ordinarily used in these experiments. Tumor No. 2129 was inoculated into twenty mice, Nos. 2184 to 2203 inclusive; tumor No. 2132 into seven mice, Nos. 2204 to 2210 inclusive, and No. 2133 into eight mice, Nos. 2211 to 2218 inclusive.

Number.	Variety of Mouse.	Age.	Death.	Weight of Tumor.
2184.....	Hybrid F_2.	36 days.	Alive 5 mos. after inoc.	0
2185.....	" "	36 "	" 5 " " "	0
2186.....	" "	36 "	" 5 " " "	0
2187.....	" "	36 "	" 5 " " "	0
2188.....	" "	36 "	" 5 " " "	0
Total....	5 Hybrids F_2.	....	....	0
2189.....	Japanese waltzing.	5 weeks.	49 days after inoc.	.610
2190.....	" "	5 "	49 " " "	.350
2191.....	" "	5 "	49 " " "	.400
2192.....	" "	5 "	49 " " "	.540
2193.....	" "	56 days.	49 " " "	.130
Total ...	5 Japanese waltzing.	....	....	2.030

EXPERIMENT 5. — *Continued.*

Number.	Variety of Mouse.	Age.	Death.	Weight of Tumor.
2194.....	Hybrid F_1.	12 weeks.	29 days after inoc.	1.700 (estimated).
2195.....	" "	12 "	49 " " "	.050
2196.....	" "	12 "	49 " " "	3.360
2197.....	" "	12 "	49 " " "	.240
2198.....	" "	12 "	49 " " "	.770
Total ...	5 Hybrids F_1.	....	....	6.120
2199.....	Japanese waltzing.	21 weeks.	49 days after inoc.	.510
2200.....	" "	Very old.	49 " " "	.450
2201.....	" "	" "	49 " " "	.100
2202.....	" "	5 months.	49 " " "	0
2203.....	" "	5 "	49 " " "	.370
Total ...	5 Japanese waltzing.	....	....	1.430
2204.....	Hybrid F_2.	35 days.	28 days after inoc.	0
2205.....	" "	35 "	28 " " "	0
2206.....	Japanese waltzing.	57 "	49 " " "	1.450
2207.....	" "	57 "	13 " " "	(Small tumor, not weighed.)
2208.....	Hybrid F_1.	54 "	49 " " "	1.540
2209.....	Japanese waltzing.	19 weeks.	49 " " "	.140
2210.....	" "	19 "	49 " " "	.115
2211.....	Hybrid F_1 (" alien ").	8 weeks.	49 days after inoc.	.780
2212.....	" " "	8 "	49 " " "	.780
2213.....	" " "	8 "	49 " " "	.410
2214.....	" " "	8 "	49 " " "	.790
2215.....	Common.	6 months.	Alive 3 mos. after inoc.	0
2216.....	"	6 "	" 3 " " "	0
2217.....	"	6 "	20 days " "	0
2218.....	"	6 "	34 " " "	0

Tumors developed in none of seven mice of generation F_2, in all six of control series of generation F_1 mice, in all seven of the young, and in six of the seven old Japanese waltzing mice inoculated. The failure of tumor to grow in one of the old Japanese waltzing mice furnishes the first negative result thus far obtained with this variety of mice. In four hybrids F^1, from the cross-breeding of a Japanese waltzing mouse with a common mouse of an "alien" stock, tumors developed, but grew more slowly than in the hybrids F_1 from similar cross-breeding of Japanese waltzing mice with the common stock first employed. The inoculation of the four common mice previously used for cross-breeding resulted negatively.

Experiment 6.

Aug. 25, 1908. Tumor No. 2114, which had been growing eighty-four days in a Japanese waltzing mouse and weighed 4.670, was inoculated into comparative series of second generation hybrids (F_2), Japanese waltzing mice, and first generation hybrids (F_1), — in all fifty-one mice. The mice were inoculated in the order of their enumeration.

Number.	Variety of Mouse.	Age.	Death.	Weight of Tumor.
2224	Hybrid F_2.	53 days.	57 days after inoc.	0
2225	" "	53 "	69 " " "	0
2226	" "	53 "	Alive 8 mos. " "	0
2227	" "	53 "	" 8 " " "	0
2228	" "	53 "	" 8 " " "	0
2229	Japanese waltzing.	28 days.	36 days after inoc.	.270
2230	" "	28 "	21 " " "	.100
2231	" "	28 "	36 " " "	.330
2232	" "	28 "	27 " " "	.550
2233	" "	28 "	36 " " "	1.030
2234	Hybrid F_1.	22 weeks.	36 days after inoc.	.795
2235	" "	21 "	36 " " "	.930
2236	" "	21 "	36 " " "	.310
2237	" "	21 "	36 " " "	.710
2238	" "	21 "	36 " " "	1.450
2239	Hybrid F_2.	37 days.	35 days after inoc.	0
2240	" "	37 "	6 " " "	0
2241	" "	37 "	37 " " "	0
2242	" "	50 "	Alive 8 mos. " "	0
2243	" "	50 "	" 8 " " "	0
2244	" "	50 "	" 8 " " "	0
2245	" "	50 "	" 8 " " "	0

Experiment 6. — *Continued.*

Number.	Variety of Mouse.	Age.	Death.	Weight of Tumor.
2246	Japanese waltzing.	26 days.	36 days after inoc.	1.500
2247	" "	26 "	36 " " "	.930
2248	" "	26 "	36 " " "	1.120
2249	" "	26 "	36 " " "	.400
2250	" "	26 "	36 " " "	.880
2251	" "	26 "	36 " " "	.655
2252	" "	46 "	36 " " "	.620
2253	Hybrid F_1.	34 days.	36 days after inoc.	.510
2254	" "	34 "	36 " " "	2.015
2255	" "	34 "	36 " " "	.465
2256	" "	34 "	36 " " "	1.270
2257	" "	34 "	36 " " "	.570
2258	" "	34 "	36 " " "	1.205
2259	" "	22 weeks.	36 " " "	.665
2260	Hybrid F_2.	38 days.	4 mos. after inoc.	0
2261	" "	50 "	Alive 8 mos. " "	0
2262	" "	50 "	70 days " "	0
2263	" "	50 "	57 " " "	0
2264	" "	50 "	Alive 8 mos. " "	0
2265	Japanese waltzing.	46 days.	36 days after inoc.	.185
2266	" "	46 "	37 " " "	.400
2267	" "	22 weeks.	36 " " "	.125
2268	" "	22 "	36 " " "	.070
2269	" "	22 "	36 " " "	.160
2270	Hybrid F_1.	22 weeks.	36 days after inoc.	.165
2020	" "	24 "	36 " " "	.785
2022	" "	24 "	36 " " "	.850
2023	" "	24 "	36 " " "	.075
2024	" "	24 "	36 " " "	.220

The mice in which tumors developed (with the exception of three) were killed thirty-six days after inoculation.

Combined weight of tumors in seventeen Japanese waltzing mice 9.335
Combined weight of tumors in seventeen hybrids F_1 .... 12.990

One of the mice of generation F_2 died six days after the inoculation, but in none of the sixteen which survived did the tumor continue to grow. In two of these, however, there was an appreciable temporary growth of the tumor, for a rounded nodule about eight millimeters in its greatest diameter developed in the three weeks following the inoculation. The nodule was subsequently rapidly absorbed in both cases. Tumors developed in all the seventeen generation F_1 mice, and in the seventeen Japanese waltzing mice of control series. In this experiment the rate of tumor growth in the young Japanese waltzing mice approximated that in old hybrids of generation F_1, and exceeds that of four others which had been previously twice inoculated with an inoculable tumor of the common mouse, so that it seems probable that in still older F_1 hybrids the rate of tumor growth would fall below that of a corresponding series of young Japanese waltzing mice. By the appropriate selection of lots of mice with reference to age as well as to variety, age, therefore, may be made to counterbalance the influence of racial susceptibility.

EXPERIMENT 7.

Sept. 9, 1908. Three small tumors, Nos. 2167, 2169, and 2171, which had attained in Japanese waltzing mice a weight of .650, .720, and .550 gram respectively in forty-eight days, were inoculated into comparative series of second generation (F_2) hybrids, Japanese waltzing mice, and first generation (F_1) hybrids — in all forty-seven mice. Six of the latter variety had previously been twice inoculated with either the Jensen or the Ehrlich tumor without result, and the other ten were derived from cross-breeding with an alien stock.

Number.	Variety of Mouse.	Age.	Death.	Weight of Tumor.
2274.....	Hybrid F_2.	26 days.	Alive 3 mos. after inoc.	0
2275.....	" "	26 "	" 3 " " "	0
2276.....	" "	26 "	" 3 " " "	0
2277.....	" "	26 "	" 3 " " "	0
2278.....	" "	26 "	" 3 " " "	0
2279.....	Japanese waltzing.	5 weeks.	Alive 4 mos. after inoc.	0
2280.....	" "	5 "	50 days after inoc.	.225
2281.....	" "	5 "	50 " " "	0
2282.....	" "	5 "	50 " " "	.350
2283.....	" "	5 "	50 " " "	1.340
2284.....	Hybrid F_1 ("alien").	11 weeks.	50 days after inoc.	1.040
2285.....	" " "	11 "	30 " " "	Small nodule.
2286....	" " "	11 "	Alive 7 mos. after inoc.	0
2287.....	" " "	11 "	40 days " "	0
2288.....	" " "	11 "	Alive 7 mos. " "	0
2289.....	Hybrid F_2.	36 days.	Alive 7 mos. after inoc.	0
2290.....	" "	36 "	" 7 " " "	0
2291.....	" "	36 "	54 days " "	0
2292.....	" "	34 "	Alive 7 mos. " "	0
2293....	" "	34 "	" 7 " " "	0
2294.....	Japanese waltzing.	5 weeks.	50 days after inoc.	.600
2295....	" "	5 "	50 " " "	.720
2296.....	" "	5 "	50 " " "	.520
2297.....	" "	5 "	50 " " "	.535
2298.....	" "	5 "	50 " " "	.065

EXPERIMENT 7. — *Continued.*

Number.	Variety of Mouse.	Age.	Death.	Weight of Tumor.
2299.....	Hybrid F_1 (" alien ").	11 weeks.	50 days after inoc.	.200
2300.....	" " "	11 "	50 " " "	.200
2301.....	" " "	11 "	50 " " "	0
2302.....	" " "	11 "	50 " " "	0
2026.....	" " "	28 "	51 " " "	.620
2303.....	Hybrid F_2.	9 weeks.	Alive 7 mos. after inoc.	0
2304.....	" "	9 "	" 7 " " "	0
2305.....	" "	58 days.	" 7 " " "	0
2306.....	" "	37 "	" 7 " " "	0
2307.....	" "	37 "	" 7 " " "	0
2308.....	" "	37 "	" 7 " " "	0
2309.....	Japanese waltzing.	5 weeks.	50 days after inoc.	.100
2310.....	" "	5 "	50 " " "	.610
2311.....	" "	5 "	50 " " "	.750
2312.....	" "	5 "	50 " " "	.180
2313.....	" "	5 "	50 " " "	.070
2314.....	" "	5 "	50 " " "	.270
2027.....	Hybrid F_1.	28 weeks.	50 days after inoc.	2.180
2029.....	" "	28 "	49 " " "	.380
2035.....	" "	22 "	51 " " "	.670
2038.....	" "	22 "	51 " " "	.190
2039.....	" "	28 "	51 " " "	.490

Comparison of growth of the tumor in the different categories of mice will be illustrated by a subsequent chart.

None of the sixteen mice of generation F_2 developed tumors. In sixteen Japanese waltzing mice there were tumors in fourteen and negative results in two. Tumors developed in five hybrids of generation F_1 of the usual stock, but the inoculation of nine hybrids of generation F_1 of an alien stock resulted in tumors in three and in a small nodule of tumor tissue in another at the time of its death thirty days after inoculation. A distinct difference is here apparent in the susceptibility of different lots of hybrids of generation F_1, which is to be accounted for by their derivation from two unrelated stocks of common mice.

Since the tumor also failed to develop in two of the Japanese waltzing mice it is possible that its virulence was somewhat diminished. Before this explanation can be accepted, however, it is necessary to consider other factors. It is not improbable that the failure of the tumor to grow in a small percentage of the young Japs was due to general malnutrition. In seven of the series of sixteen the body weight diminished instead of increased and the gain in the other nine was slight. It appears that, on account of a misunderstanding, sufficient nourishment had not been provided these mice, for when killed they presented no evidence of disease, and being so young they should have shown a considerable increase in weight during the period of the experiment. Tumors in some cases develop notwithstanding loss of weight, as may be seen not infrequently in individual instances, but such a condition of malnutrition is to be considered less favorable to the development of the tumor. The low percentage of tumors in the alien hybrids may be attributed to a greater degree of insusceptibility on the part of the common parent or a less degree of susceptibility on the part of the Japanese waltzing parent. In addition to this the series of mice was slightly older than the series of alien hybrids in Experiment V., and, on account of the death of several from this cage, they evidently had some infectious disease, the nature of which was not determined.

In order to determine if by any possibility the susceptibility to the inoculable tumor of the Japanese waltzing mouse reappeared in the next generation (F_3), the following two experiments were performed. Comparative series of Japanese waltzing mice, and hybrids of generations F_1, F_2 and F_3 were inoculated.

EXPERIMENT 8.

Nov. 13, 1908. Tumor No. 2346, which weighed .920 gram after growing forty-three days in a Japanese waltzing mouse was used to inoculate several series of mice, including those of the third generation (F_3), with the following results:

Number.	Variety of Mouse.	Age.	Result of Inoculation.
2389	Hybrid F_3.	17 days.	O
2390	" "	17 "	O
2391	" "	17 "	O
2392	Hybrid F_2.	31 days.	O
2393	" "	31 "	O
2394	" "	31 "	O
2395	Hybrid F_1.	6½ months.	+
2396	" "	6½ "	+
2397	" "	53 days.	O
2398	Japanese waltzing.	30 days.	+
2399	" "	30 "	+
2400	" "	30 "	+
2401	Japanese waltzing.	2½ months.	+
2402	" "	2½ "	+
2403	" "	2½ "	+

None of the mice of generation F_2 or F_3 developed tumors. All the Japanese waltzing mice developed tumors, as well as two of the three mice of generation F_1. This hybrid in which the tumor failed to grow had for one parent one of the common females which had been used in previous breeding, but for its other parent it had a Japanese waltzing male which had not been bred from previously. The mother sickened soon after the young were born and only this one survived from the entire litter.

EXPERIMENT 9.

Dec. 31, 1908. Thirteen third generation (F_3) hybrids and fourteen waltzing mice were inoculated with tumor No. 2398, which weighed .650 gram after growing forty-eight days in a Japanese waltzing mouse.

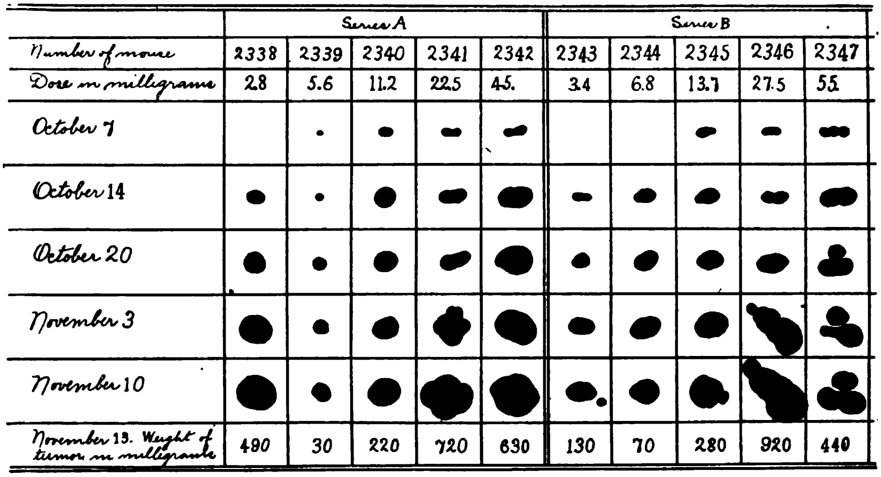
Number.	Variety of Mouse.	Age.	Result of Inoculation.
2430	Hybrid F_3.	65 days.	0
2431	" "	65 "	0
2432	" "	63 "	0
2433	" "	63 "	0
2434	" "	60 "	0
2435	" "	60 "	0
2436	" "	38 "	0
2437	" "	38 "	0
2438	" "	38 "	0
2439	" "	23 "	0
2440	" "	23 "	0
2441	" "	23 "	0
2442	" "	23 "	0
2443	Japanese waltzing.	60 days.	+
2444	" "	60 "	+
2445	" "	60 "	+
2446	" "	60 "	+
2447	" "	60 "	+
2448	" "	60 "	+
2449	" "	60 "	+
2450	" "	60 "	+
2451	" "	60 "	+
2452	" "	60 "	+
2453	" "	60 "	+
2454	" "	60 "	+
2455	" "	4½ months.	+
2456	" "	4½ "	+

In Experiment 9, fourteen Japanese waltzing mice and thirteen mice of generation F_8 were inoculated with result that the tumor grew in all of the former and in none of the latter series.

The following experiment was performed for the purpose of determining the influence of dosage * in the transplantation of the Japanese waltzing mouse tumor. The results, although too meager to allow of generalization concerning dosage, nevertheless furnish additional data concerning racial susceptibility.

EXPERIMENT 10.

Oct. 1, 1908. A portion of tumor No. 2266 was weighed, and then divided as nearly as possible into two equal halves. One of these halves served as the largest dose; the other half was again divided into two equal portions, and one of these was taken as the next dose. In this manner series of doses were obtained, each approximately of one-half the weight of the one preceding. In each series the doses were inoculated in the order of their size, the smallest dose first. The tumor tissue was deposited as near as possible in the same situation in each mouse, and the same technical procedure was followed in each case. The mice were of the same age and of approximate weight, but the two series were comprised of more than a single litter. The mice were killed and the tumors weighed forty-three days after the inoculation.



	Series A					Series B				
Number of mouse	2338	2339	2340	2341	2342	2343	2344	2345	2346	2347
Dose in milligrams	2.8	5.6	11.2	22.5	45.	3.4	6.8	13.7	27.5	55
October 7		●	●	●	●			●	●	●
October 14	●	●	●	●	●	●	●	●	●	●
October 20	●	●	●	●	●	●	●	●	●	●
November 3	●	●	●	●	●	●	●	●	●	●
November 10	●	●	●	●	●	●	●	●	●	●
November 13. Weight of tumor in milligrams	490	30	220	720	630	130	70	280	920	440

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* The influence of dosage and of "concomitant immunization," a closely related factor, are taken up very thoroughly in the Third Report of the Imperial Cancer Research Fund, which appeared while the present investigation was in progress.

It is apparent from the accompanying chart that there is in these two series some correspondence between the amount of tumor tissue inoculated and the size of the resulting tumors at any given time. That is, if the series are divided arbitrarily, it will be seen that tumors of greater weight have been produced by the larger doses (*i.e.*, those on the right hand side of each of the two series shown in chart). It will be seen also that the correspondence of the size of the tumors to the size of doses used is rather more marked at the time of the earlier observations. As the tumors continue to develop discrepancies appear, which are to be explained only by differences of soil, as provided by the tissues of the different individuals of the series. In both series the smallest dose resulted ultimately in a larger tumor than did a dose of twice its size. Certain differences which are apparent for a time disappear later on. Thus it seems probable that, if these tumors were allowed to develop for a long time, the effect of dosage would be counterbalanced by individual variation in the soil furnished the tumor. On the other hand, it is not improbable that, if larger numbers of animals were used in experiments of this sort, the combined results would be influenced by dosage. If consideration is given to the percentage increment in weight as well as to the absolute increase in tumor tissue after implantation it will be observed that there is little correlation between dosage and growth. Thus in the first series the smallest dose produced in forty-three days a tumor one hundred and seventy-five times its weight, while the largest dose produced a tumor only fourteen times its weight. It does not hold throughout the series, however, that the coefficient of growth is greater with the doses of small size. Thus the second smallest dose of the same series produced a tumor of only five and one-third times its weight.

The ten Japanese waltzing mice inoculated in this experiment all developed tumors, although some received very minute and others relatively large doses of tumor tissue. Both large and small doses are thus found to be effective.

The object of the following experiment was to determine if the growth of the tumor in hybrids of the first generation modified it in any way so that it would grow in subsequent generations.

EXPERIMENT 11.

Dec. 31, 1908. Eight hybrids of generation F$_2$, and two control Japanese waltzing mice were inoculated with tumor No. 2259, which had grown somewhat over four months in a first generation hybrid, having recurred after an attempt at removal.

Number.	Variety of Mouse.	Age.	Result.
2420	Hybrid F$_2$.	67 days.	o
2421	" "	67 "	o
2422	" "	67 "	o
2423	" "	40 "	o
2424	" "	40 "	o
2425	" "	40 "	o
2426	" "	40 "	o
2427	" "	40 "	o
2428	Japanese waltzing.	4 months.	+
2429	" "	" "	+

The tumor by growing for a period of time in mice of the first generation (F$_1$) is not so modified as to enable it to grow in mice of the second generation (F$_2$). The results are similar to those in the experiments in which the tumors grown in Japanese waltzing mice were used. In another instance the inoculation of common mice with a tumor which had grown in a first generation hybrid was unsuccessful.

The results of all these experiments, together with those of all other inoculations of the Japanese waltzing mouse tumor, are combined in the following table:

RESULTS OF ALL INOCULATIONS OF THE JAPANESE WALTZING MOUSE TUMOR.

	Common Mice.		Japanese Waltzing Mice.		Hybrids, Generation F_1.		Alien Hybrids, Generation F_1.		Hybrids, Generation F_2.		Hybrids, Generation F_3.	
	+	−	+	−	+	−	+	−	+	−	+	−
Transplant I..		....	3									
" II..		....	(2).....		One died One grew		within a sh tumor on re		ort time. inoculation.			
" III..		....	(1).......		Grew tum		or on reinoc		ulation.			
" IV..		2	1									
" V..		7	4									
" VI..		....	4									
" VII..		....	3									
" VIII..		....	6									
" IX..		....	8									
" X..		6	3	Inocu	lated with		tumor from		Hybrid F.			
Experiment 1..		10	10		11							
" 2..		12	12		22							
" 3..		7	7		....		....			6		
" 4..		....	5		5		....			5		
" 5..		4	13	1	6		4			7	Failure in old Jap.	
" 6..		....	17		17		....			17		
" 7..		....	14	2	6		4	5		16		
" 8..		....	6		2	1	....			3		3
" 9..		....	14		....		....		....			13
" 10..		....	10									
" 11..		....	2	Inocu	lated from		Hybrid F_1.			8		
Total.......		48—	142+	3—	69+	1—	8+	5—		54—		16—

The results may be summarized as follows: A racial peculiarity, *i.e.*, susceptibility to an inoculable tumor, appears in the first generation (F_1) obtained by cross-breeding a susceptible with an insusceptible race of mice. This peculiarity occurs in hybrids of both common and Japanese waltzing mothers, so that it is transmitted by the male as well as by the female parent. In the next generation (F_2) of mice, produced by the breeding inter se of the hybrids (F_1), all were like common mice insusceptible. The appearance of a peculiarity in the first generation resulting from the cross-breeding of two different varieties of animals and its total disappearance in subsequent generations I am unable to accord with Mendel's law or any other principle of heredity as yet discovered. After having found the development of the tumor to be more rapid in the hybrids obtained by cross-breeding an insusceptible with a susceptible mouse than in their susceptible parent, we are then confronted with the almost incredible results in that these hybrids, of which very nearly one hundred per cent are proven susceptible to the tumor, when bred together produce offspring none of which (0 per cent) are susceptible. A certain proportion of the mice of the second and third filial generations manifest the waltzing character, but are nevertheless insusceptible. Thus we have the hybrids of the first generation, which from their appearance alone cannot be distinguished from common mice, susceptible to the tumor of the Japanese waltzing mouse, and waltzing mice of the second and third generations, which are all insusceptible.

On account of the peculiarity of these results, it is necessary to consider as far as possible all the conditions which could in any way be of influence. The method of inoculation employed has been described already, and the same method was used both in the series of mice to be tested and in the control series. Whether, by the inoculation of much smaller or of much larger doses, the tumor could be made to grow in mice of the second or third filial generations has not been ascertained. The comparative series of mice were reared in essentially the same environment, since they were kept in the same room, and were fed with food prepared in common for all. Before the experiments were ended bacterial infections were noted in certain individuals, but these occurred both in the mice which proved susceptible as well as in those which proved refractory. Change of conditions dependent upon the lapse of time necessary in the breeding of the successive generations does not account for the difference in their susceptibility, for certain of the generations to be compared were reared and tested simultaneously. The mice of generation F_8 and a portion of those of generation F_2 were reared from parents which had already been inoculated. Other observations with other inoculable tumors indicate that the inoculation of the parent does not render subsequent offspring immune. In generation F_2 the susceptibility of fifty-four mice was tested. Of these, thirty-three were of uninoculated parents and twenty-one were of parents either one or both of which had been inoculated. Even, if the latter are excluded in the consideration of the result, the number left is sufficient to base conclusions upon. Thus, after close analysis, the almost absolute difference in the susceptibility of the different generations to the tumor must be considered as the result of cross-breeding a susceptible with a non-susceptible variety of mice rather than of adventitious influences.

That identical results might not be obtained if other stocks or varieties of mice were used is indicated by the partial rather than absolute susceptibility of the "alien" hybrids inoculated in Experiment 7.

The fact, however, that mice which show the greatest possible susceptibility to an inoculable tumor produce young which are in every case insusceptible indicates the necessity of taking the racial factor into account in practically all investigations of the inoculable tumors. The failure to recognize the influence of racial differences leads to erroneous conclusions with the use of large as well as small numbers of animals. Unless the susceptibility of the stock of mice employed has been previously ascertained by repeated inoculations each experiment must be carefully controlled, otherwise the results obtained are unreliable. The uncertainty of the character of mice purchased in the open market must always be taken into account. Certain investigators have controlled their experiments by dividing a lot of mice into two series, one designed to control the other. It is obviously unsafe to compare results in different lots obtained from the dealers. It would appear that, in order to adequately control certain experiments, it would be necessary to take comparative series from the same litter of animals.

Results such as those already outlined have a direct bearing on the question of the so-called "Fluctuations" or "Rhythms" in the growth of the transplantable tumors of mice. The subject has been taken up at a considerable length both by Calkins and by Bashford and his collaborators. In that which has been rather loosely termed the "virulence" of tumors, Calkins[12] distinguishes two distinct factors, the property of the tumor which he terms "infectivity," as indicated by the percentage of the positive results following its inoculation, and the time factor (rate of growth) in the development of tumors. He criticises Bashford's previous work in that he has taken the "infectivity" of tumors as an index of their energy of growth. He endeavors to show that there is no correlation between the two. One of the two charts used by Calkins to illustrate this point shows a fairly uniform correlation of the percentage of successful results with the estimated rapidity of growth, so that his contention is not proven by the data which he collected. In the construction of his chart for the comparison of infectivity with the growth energy of tumors Calkins takes as a measure of the latter the average time in days required by the tumors of a given generation to kill the animals in which they are growing. It is obvious that the opportunity for error in such a method of estimating the energy of tumor growth is so great that the reliability of any conclusions which may be derived therefrom is to be questioned. Death may be the result of conditions either wholly independent of, or secondary to the growth of the tumors. Racial differences in the lots of mice used in the consecutive transplants are apparently not considered, and beyond stating that he is unable to account for rhythmic variation in the activity of tumors by the inoculation on different occasions of more or less susceptible animals, — *i.e.*, animals of different ages, — no further comment is made concerning this factor. From the study of growth energy estimated by the periods required for the tumors to kill, Calkins concludes that there is no evidence of a rhythmic development such as that which is found in early embryonic cleavage or in free living organisms such as the protozoa, but that rhythms of tumor growth, if they occur, are probably to be found in the individual mouse. Variation in the infectivity of a given tumor he thinks is possibly due to the stimulus of a foreign organism, which may be either ultra-microscopic or unrecognizable, rather than to an inherent property of the tumor cells. He states that the spirochetes which are often found in mice with tumors may account for differences in the percentage of the successful results, possibly from the reason that they in some way prepare the soil for the tumor cells.

Bashford,[13] and later Bashford, Murray, Haaland, and Bowen,[14, 15] on the other hand, have taken the ground that the percentage of successful results attending the inoculation of a tumor furnishes an index of the energy of growth. They explain that this method of measurement is a purely arbitrary one, and that it was selected on account of its convenience of application. The fact is recognized that if a tumor should be obtained, the transplantation of which was uniformly successful (one hundred per cent), any fluctuations in the energy of growth could not then be estimated by the method adopted. Bashford, Murray, and Bowen, in taking up "The experimental analysis of the growth of cancer," consider the following factors which influence the rate and amount of tumor growth: race and age of the mice employed, site of implantation, dosage and method of inoculation, and variations in the character of the tumor. Apart from the matter of race, due attention appears to have been paid to the influence of these various factors. It is stated that mice of the same race were used, and also that varieties prized by mouse fanciers were avoided as unsuitable. Just what is indicated by "race" is not apparent. If it is meant that the mice used were similar in appearance or that they were obtained from one or from several dealers of a given locality, but little is signified by the term "race." In the present investigation, invisible racial differences are found to be of such importance with reference to variations in the growth of tumors that the exact origin of all mice used in the successive transplants should be determined. The necessity of this is not overcome by the employment of large numbers.

The graphic method employed by Bashford and his collaborators, by which the percentage of successful results is depicted by a branching curve of the nature of a genealogical tree, is of considerable value in presenting the results of successive transplants. By the construction of what is termed the " main stem " of the curve through the selection in each generation of the highest percentage of successes obtained, it is shown that the highest percentage of successful results may steadily arise until a maximum is reached, and that this is often followed by a sudden drop. It seems not improbable that selection plays a part in the attainment of the high percentage of successes, although it is shown that descending branches may subsequently also arise to a high point. The fact that, in a large number of the generations of tumor depicted, a rise in the "implantability" of certain tumors is offset by a lowering of this in others is not in accordance with the hypothesis expressed by these investigators that the fluctuations in the activity of the tumor are due to some inherent property of the tumor tissue. Thus in the graphic records both ascending and descending branches are found in a given generation of a tumor, and only rarely are all the branches directed upward. They, however, would possibly explain such differences in the activity of the tumors of a given generation either by differences in the period of their development before inoculation or by variation in the cells of the tumor from which they were derived. It seems probable that such differences are to be explained in part at least by other factors, such as varying degrees of susceptibility in the lots of mice used.

The conclusions reached by these authors are that secondary biological alterations of the character of the inoculable tumor may accompany the adaptation of its cells to growth in a strange individual. The accelerated rate of growth in a propagated tumor may be a result of long continued selection of the most rapidly growing tumor tissue. Environment is also to be considered from the fact that tumors growing in the mice of one country are transplanted with difficulty to the mice of a foreign country. Evidence is found, however, of periodic changes in the activity of tumor epithelium, and " that the duration of the alternating phases of increased and diminishing energy of growth is an inherent character of the parenchyma cells of the same order of permanence as the histological arrangement in which they grow."

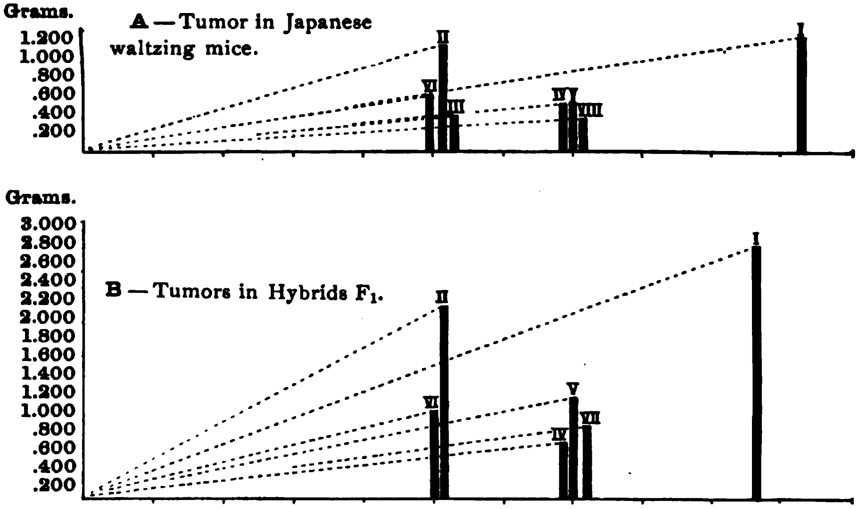
Variation in the rate of growth of transplanted tumors must be accepted as an established fact. A marked difference in the rate of growth of the first as compared with that of subsequent generations of a transplanted tumor is found almost invariably. From the fact that the tumor, employed in the greater part of this investigation, usually grows in one hundred per cent of the Japanese waltzing mice inoculated, it is apparent that the number of successful results cannot be taken as an index of the rate of tumor growth, as Bashford and his collaborators have done. Although it is probably true that those inoculations which yield the highest percentage of successful results usually also yield the most rapid rate of tumor growth, it is questioned whether the ability of the tumor cells to adapt themselves to a new soil is to be taken as an accurate measure of their rate of growth. The method employed by Calkins of estimating the energy of growth by the time necessary for the tumor to kill the animal is also inaccurate.

On account of the necrosis of a variable proportion of the tumor tissue implanted, the amount of living tissue with which the tumor commences its development, which has been termed by Bashford, Murray, Haaland, and Bowen " the effective initial dose," cannot be accurately estimated.

The above-named authors show that in certain strains of tumors the size attained is proportionate to the size of the doses employed. With other strains the reverse is found to be true, and small doses produce more tumors and larger tumors. The character of a tumor may vary, and, whereas large doses give at one time a higher percentage of rapidly growing tumors, at another they result in a spontaneous absorption of the tumors which develop. Thus it would appear that the injection of equal doses in the successive transplants of a tumor does not assure the equality of the actual amounts of tissue with which the tumor commences. With many tumors, therefore, no more than approximate uniformity of dosage is possible. The results obtained in Experiment 9 indicate that dosage in the case of the relatively slow-growing, Japanese waltzing mouse tumor is of less importance than the character of the animal into which the tumor is inoculated. Approximately equal amounts of living tumor were, however, inoculated in all comparative series of mice. Since it is impossible to weigh the tumors at intervals during their growth the construction of a curve showing the percentage increments in weight is practically out of the question. They may be measured and charted by the graphic method frequently employed, but this method lacks accuracy, especially in tumors of small size, and in my own experience I have found that such estimates of the relative sizes of a series of tumors correspond only roughly with their actual weights. The various generations of a tumor may be weighed after certain definite periods of growth, and compared with the average weight attained by tumors of other generations. By this method the average daily increase in weight may be estimated for the tumor's development in a single animal or lot of animals with more accuracy than by any method which has been used.

In the accompanying chart (Text Fig. 1) the average weights attained by the tumors in seven consecutive transplants are illustrated by the heights of the black columns, and the time taken in development is shown by the position of the columns on the base line. It is at once apparent that the tumor in one transplant may attain in thirty-six days about three times the weight attained in fifty days in another transplant. It is to be noted that the average weight of the tumors is much higher in the hybrids than in the Japanese waltzing mice in each transplant, and also that variation apparent in the former corresponds more or less closely with variations in the latter. The average daily increase in the weight of the tumors may be estimated by dividing the average weight by the number of days for which they were developing.

FIG. 1.



Average weights attained by the tumors of seven consecutive transplants indicated by heights of black columns. Time taken in development shown by position on base line, each division representing a period of seven days.

With regard to variations in the average increase in weight per day, there is in these generations of the tumor more or less correlation between hybrids and Japanese waltzing mice. On investigating the ages of the various lots of mice, it was found that with few exceptions the growth of the tumor was retarded when it was inoculated into older mice and accelerated when inoculated into younger mice. The influence of age is quite apparent when lots of different ages of the same variety are inoculated simultaneously (see Text Fig. 2). Haaland's observations that inoculable tumors develop with greater uniformity in the young than in the old are confirmed by these experiments. It cannot be assumed, however, that differences in the rate of growth in the successive generations of the tumor are dependent solely upon differences in the age of the animals inoculated. Other factors, such as the condition of the tumor at the time of its inoculation, undoubtedly influence the results.

In this series of experiments, those generations of the tumor which showed exceptionally rapid growth resulted from the inoculation of tumors which had grown for a relatively long period of time (seventy-six days) in a single animal. Other generations produced by the inoculation of tumors which were taken after a short period of growth (twenty-one or twenty-four days) showed a much slower rate of growth. The influence of personal equation in the selection of the tumors to be inoculated has been already mentioned. At one time a rapidly growing tumor and at another time a tumor which had been growing slowly for a long time may suit the convenience of the investigator. There is ample evidence to show that the character of a tumor as regards its power of growth becomes modified by continuous transplantation. The differences in the growth of the first as compared with subsequent transplants have been repeatedly noted. This modification is probably due to changes in the tumor's environment rather than to an inherent tendency of its cells.

In addition to those conditions already discussed, such as the age of the inoculated animals, the method of inoculation, the condition of the tumor at the time of inoculation, etc., there are other varying conditions to which but little attention has been given. The periodical activities of certain tissues of the host in which the tumor grows may influence the growth of the latter. Haaland has noted the retarding influence of pregnancy on the growth of mouse tumors. The activities of the mammary gland and the ovary are periodical. Such conditions, however, affect individuals rather than lots of mice. The shedding of the fur is periodical in mice, and it may occur simultaneously in large numbers of mice. The manufacture of so great an amount of fur within so short a period of time is brought about by a sudden growth of the epithelium of the hair sheaths, which probably surpasses in rapidity the growth of many of the malignant tumors. It is not improbable that the growth of the implanted tumors may be affected by such marked variation in the activity of tissues of the inoculated animal. Seasonal changes, with variations of temperature producing changes in the vascularity of the skin, and conditions influencing the general nutrition of the animals may be of great importance. In Experiment 7 the low rate of growth may be due largely to the fact that the animals were underfed. Haaland has suggested that changes in food, and possibly climate, resulting from the transportation of mice from Germany to Sweden may modify the susceptibility of mice, and it does not seem improbable that such changes might influence the rate of growth of tumors. The growth of tumors appears to be modified by associated bacterial infection, and, as Calkins suggests, it may possibly be influenced by an infection with an unrecognized organism.

Thus, although there is no difficulty in establishing the occurrence of variation in the rate of tumor growth, the dependence of this either wholly or in part upon an inherent property of the tumor cells does not appear to be proven. The environmental conditions influencing the growth of transplanted tumors are numerous, and it is obviously impossible to obtain identical conditions in successive experiments. Among those recognized factors which influence the growth of tumors, differences of race or, better, of blood relationship are of great importance. It has been demonstrated in the foregoing experiments that such inherited differences in susceptibility over and above all other varying conditions account for fluctuations in the growth of the tumor as great as those which are ordinarily found in successive generations. If the tumor which is growing in Japanese waltzing mice is inoculated into the first generation of hybrids, obtained by cross-breeding with insusceptible common mice, its growth is thereby greatly accelerated. If transferred back to waltzing mice its growth is retarded. The breeding together of highly susceptible parents does not ensure susceptible offspring, for notwithstanding the great susceptiblity of the first generation of hybrids their offspring proved to be all insusceptible. Thus the rate of tumor growth may be varied at will. It is possible that, by appropriate selection of lots of mice with reference to age as well as variety, age could in some cases be made to counterbalance the influence of racial susceptibility.

This might also prove true of dosage, if it were possible to determine the most effective dose for each transplant. Slight variations such as occur in these latter factors in the foregoing experiments are insufficient to overcome the influence of race. Not only are racial differences important with regard to susceptibility, but it is also possible to go further, and account for much of the variation by individual or family differences. Thus it has been observed that the growth of the tumor is much more rapid in hybrids obtained from one pair than in those from another pair, although in both pairs the respective parents were taken from a single family of Japanese waltzing mice. Even with closely related mice, therefore, there is considerable variation with regard to the character of the soil furnished for the growth of the tumor.

Heretofore attention has been directed chiefly to variations in the growth of different generations of the tumor. The fact that the different generations are produced by the inoculation of different individual tumors, which have been more or less modified by peculiarities in the environment furnished by the tissues of the animal in which they have grown, should be taken into consideration. Not only is the character of the soil upon which tumors are implanted of importance, but also differences in the properties of the individual tumors inoculated. In the experiments here presented, comparative series of different varieties of mice were inoculated simultaneously with a single tumor. The constant differences found in the rate of tumor growth in respective series of mice cannot in this case be ascribed to any difference in the character of the tumor tissue inoculated.

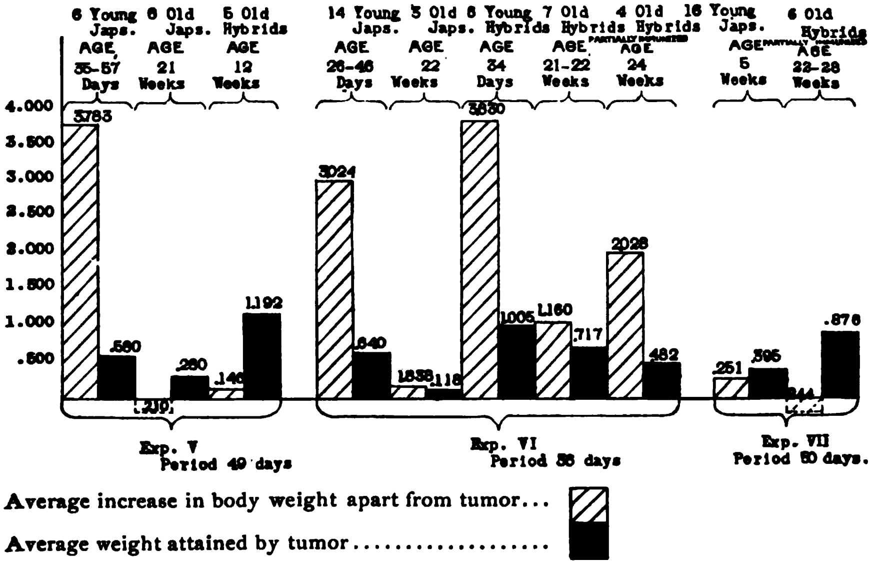
The error involved in the comparison of the development of the tumor in different generations is thereby avoided.

The unsuccessful inoculation of common mice with the Japanese waltzing mouse tumor affords no protection against subsequent inoculation with the Ehrlich tumor. Eight common mice, which in Experiment I had been inoculated with the Japanese tumor, were inoculated ten weeks later with the Ehrlich tumor, with the result that large rapidly-growing tumors developed in all. Likewise the inoculation or even the transient growth of the Jensen or of the Ehrlich tumor does not protect first generation hybrids against the Japanese waltzing mouse tumor. The latter tumor grew to large size in each of seven hybrids which had been twice inoculated (Experiments E and F) with either the Jensen or the Ehrlich tumor, although these tumors had grown appreciably after the first inoculation.

In an attempt to account for the rapid growth of tumors in the hybrids of the first generation, it was thought that perhaps the greater size of these mice or the rapidity of their growth might be of influence. The ultimate size attained by a mass of tumor tissue is obviously dependent to a large extent upon the size of the animal in which it grows. In each experiment the first generation hybrids weighed much more than the Japanese waltzing mice. If, however, mice of the same series or even of the same litter were compared, no correlation was found between the weight of the animal and the weight of the tumor. The larger tumors were as often found in the smaller as in the larger animals of a given litter. Thus in its early development at least the size of the tumor is in no wise dependent upon the relative size of the animal. Whether, in the latter period of their development, the tumors would grow more rapidly in large than in small animals was not ascertained.

It was thought possible that the more rapid growth of the first generation hybrids, as compared with Japanese waltzing mice of similar age, might account for the more rapid development of the tumors in the former. By weighing the mice at the beginning and at the end of the experiment the increase in the weight of the body apart from the tumor was determined. The body growth is invariably greater in hybrids than in Japanese waltzing mice of similar age. If, however, practically full grown hybrids are compared with young rapidly growing Japanese waltzing mice the tumors nevertheless are on the average larger in the hybrids. In a given variety of mice the tumor develops more rapidly in those series in which the body growth is greatest, — that is in young animals, — but the rate of body growth cannot be taken as a basis upon which to explain the greater growth of the tumor in hybrids. Since it develops more rapidly in hybrids than in Japanese waltzing mice irrespective of increase in body weight, it appears that there is a qualitative rather than a quantitative difference in the soil furnished the tumor by these two varieties of mice. In the following chart the average weight attained by the tumors together with the average increase in body weight is compared in different series of mice in three experiments.

FIG. 2.



It is not at all certain whether the rapidity of tumor growth in young animals is dependent upon the rate of body growth or upon other conditions associated with youth. It is not uncommon to get rapidly growing tumors in old individuals, although usually it is slower than in the young. Such individual variations may be acquired, therefore, during extra-uterine life. Tumors may continue to develop notwithstanding decrease in body weight. In one experiment five mice were inoculated at the same time with the same tumor, and tumors developed in all. One grew progressively more and more emaciated, and it was finally found that the animal had been gradually starving on account of a deformity of the teeth, which interfered with eating. The tumor of this mouse was much smaller than those of the other four, but it had continued to grow notwithstanding a loss of weight on the part of the rest of the body.

The histological character of the Japanese waltzing mouse tumor is not modified in any way by transplantation into first generation hybrids. The tumors in general show less necrosis, and the tissues surrounding the tumor are considerably more vascular in the hybrids than in Japanese waltzing mice, so that it seems not improbable that a more active vascular reaction on the part of the hybrid may account for the more rapid growth of the tumor in them.

A study of the histological changes concerned in the reaction of the non-susceptible mice of the second generation (F_2) has been made, but this subject will be taken up in a separate paper.

The results of this investigation have led to the following conclusions:

The successful transplantation of tumor tissue in mice is dependent upon three main factors, — the method of inoculation, the character of the individual tumor employed, and the nature of the soil upon which the tumor is implanted. All three are subject to great variation, as are also the numerous subsidiary conditions upon which they are based.

With regard to the nature of the soil or environment furnished the implanted tumor cells by the tissues of the host, racial differences are especially important. Although it is possible that susceptibility to inoculable tumors may be affected at times by external conditions, the occurrence of distinct differences in the susceptibility of lots of mice bred and kept under identical conditions shows that susceptibility may be of the nature of a racial peculiarity. As such it is transmitted from generation to generation of imbred varieties or races of mice. The insusceptibility of a race of mice to a given tumor may be either relative or absolute, and relative racial insusceptibility may be augmented by the inbreeding of the more insusceptible individuals.

A tumor which originated in a Japanese waltzing mouse has been transplanted for many generations in mice of the same variety, but invariably fails to grow when inoculated into common mice. Attempts to inoculate Japanese waltzing mice with the Jensen tumor have been unsuccessful, and the Ehrlich "Stamme II." tumor in this variety develops more slowly and in fewer cases than in common mice.

The susceptibility of hybrids, resulting from the cross-breeding of common albinos with Japanese waltzing mice — each either relatively or absolutely insusceptible to the tumors of the other — has been ascertained.

The hybrids of the first generation (F_1) were found to be slightly more susceptible than Japanese waltzing mice, but less susceptible than common mice to the tumor of common mice. The Japanese waltzing mouse tumor, on the other hand, grows more readily in these hybrids than in Japanese waltzing mice. The offspring of such hybrids are found, however, to be absolutely insusceptible to this tumor, as are mice of the third filial generation. Susceptibility to an inoculable tumor is neither, therefore, inherited in accordance with Mendel's law, nor are the results obtained from cross-breeding explained by any other known principle of inheritance. The number and the uniform character of the results are regarded as adequate basis for this conclusion. Whether the facts presented concerning the elimination of a racial peculiarity as the result of cross-breeding will be found to correspond with any generally applicable law remains to be answered by future observation.

There is no correlation between any of the visible racial characters and susceptibility to the inoculable tumors employed. Thus the hybrids of the first generation outwardly resemble common mice, but are nevertheless susceptible to the Japanese waltzing mouse tumor, whereas the waltzing mice which appear in subsequent generations are insusceptible to the same tumor.

It is possible that results of another sort may attend the cross-breeding of other varieties or breeds of mice, but from the data at hand it is apparent that the factor of race or, better, of blood relationship is of considerable importance in the investigation of transplantable tumors. In each of the foregoing experiments the racial element has outweighed all minor variations in associated conditions. It seems probable that even slight racial differences may account for a large proportion of the fluctuations frequently noted in the so-called "infectivity" of tumors and in the rate of tumor growth.

The methods heretofore proposed for the measurement of the rate of tumor growth are inaccurate, and for its estimation the exact weight or volume of the tumor as well as the period of growth must necessarily be ascertained.

Many of the variable conditions which possibly influence tumor growth have apparently not been investigated. The activities of certain of the tissues of the inoculated animals are periodical. An instance of this is to be seen in the activity of the hair follicles at the time of the shedding of the fur.

It is found that the size of an animal does not influence the rate of growth of a tumor, except possibly in its late development.

Tumors grow most rapidly in young animals in which the body growth is greatest. This does not explain the more rapid growth of the Japanese waltzing mouse tumor in hybrids, however, and the difference between the soil furnished by such hybrids and that furnished by Japanese waltzing mice appears to be a qualitative rather than a quantitative one.

The histological character of the tumor is not modified in any respect by its implantation into hybrids, neither is it thereby adapted for growth in the mice of subsequent generations or in common mice.

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THE NATURE OF THE REACTION OF THE TISSUES OF SUSCEPTIBLE AND NON-SUSCEPTIBLE MICE TO AN INOCULABLE TUMOR.*

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It has been observed repeatedly that in mice there exist racial differences in susceptibility to inoculable tumors. Such differences in susceptibility have been noted in the case of mice inoculated with an adenocarcinoma which originated spontaneously in a Japanese waltzing mouse (Tyzzer, E. E., p. 193). Implants of this tumor have been observed to grow in ninety-eight per cent of Japanese waltzing mice inoculated, while common mice have proved in every case non-susceptible. Furthermore, as a result of cross-breeding susceptible Japanese waltzing mice with common mice, it has been found that hybrids of the second filial generation are in all cases non-susceptible to the tumor.

The present investigation consists of a study of the histological basis of this difference in susceptibility. Japanese waltzing mice have been used throughout as the susceptible animals, and hybrids of the second generation as the non-susceptible. Series of susceptible and non-susceptible mice have been inoculated, killed at certain periods after inoculation, and the tissue taken for histological study.

With regard to the inoculability of mouse tumors in general it was shown by Jensen,[1] — by the examination of the "early stages" in the development of transplanted tumors,— that the parenchyma of the new tumor is derived entirely from the cells of the parent growth. Following this, Bashford and Murray,[2] working with Jensen's tumor, showed that the connective tissue and vascular supply of the successfully implanted cancer are derived solely from the tissues of the new host. They find that the injected mass is surrounded by a fibrinous exudate within two hours after inoculation,

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and that within the first fifteen hours there are present aggregations of polymorphonuclear leucocytes which have penetrated the interstices of the tumor tissue, especially those portions which are necrotic. At this time (fifteen hours) they describe the beginning of proliferation by the amitotic division of the surrounding connective tissue cells of the host. This process continues until the tissue around the injected material becomes very cellular. The fibroblasts thus formed by direct or amitotic division subsequently undergo division by mitosis and penetrate the tumor mass. Concomitant with this their preparations show a retraction and gradual degeneration of the introduced stroma. By the end of four days they note the commencement of the growth of capillaries into the tumor mass; and this continues until finally the growth has acquired a new blood supply which, like the connective tissue elements of the stroma, is derived entirely from the tissues of the new host.

While the present investigation was in progress a histological study of mouse tumors by Russell[3] appeared. This included a comparison of the early stages of inoculated carcinomata in normal mice and in mice rendered resistant by the previous injection of mouse blood, mouse embryo emulsion, or normal mouse tissues. Injections of this sort had already been shown to render such mice immune to tumors subsequently inoculated,—Bashford, Cramer, and Murray,[4] and Shöne.[5] In addition to these, mice which had once proved resistant to an inoculation with the tumor were also used for comparison with susceptible mice, and the results were found to be similar to those observed in mice artificially immunized. This fact led Russell to conclude that the nature of the immunity was identical in both. He reports in detail his observations upon the early stages of an inoculable adenocarcinoma of mammary origin which gave an average of about thirty per cent of successful implantations. A study was also made of the early stages in the development of other inoculable tumors, including the Jensen tumor. The changes were essentially similar in all the tumors used. Russell's principal finding was a complete absence of stroma formation in the resistant mice. He observed in these animals that the implanted parenchyma excited no formation of fibrous or vascular stroma on the part of the tissues of the host, and soon became necrotic, with the exception of those cells at the periphery of the masses which lay in contact with the tissues of the host. This epithelium, he noted, continued its growth for several days, and usually formed a small cyst, but was finally destroyed apparently by the late ingrowth of fibrous tissue. A similar study of these tumors, inoculated into rats, showed, on the other hand, a perfect "stroma reaction" on the part of the tissues of the rat, a vigorous growth for about nine days, and then a simultaneous degeneration of all living tumor cells, with a subsequent complete absorption of the growth. Thus from his observations it appears that in mice the failure of growth is due to absence of stroma formation on the part of the host's tissues, while in rats the ultimate destruction of the tumor occurs after the growth has received a stroma, and is the result of a simultaneous degeneration of all the introduced parenchyma.

In the following experiments the changes resulting from the inoculation of the Japanese waltzing tumor into susceptible and into non-susceptible, or refractory mice, have been the object of study. This tumor originated as a primary adenocarcinoma in a Japanese waltzing mouse, and has been described in a previous paper by Tyzzer.[6] In its present state of growth it consists of solid masses of epithelial cells with a relatively small amount of stroma. It extends by the continuous invasion of its capsule by processes of tumor tissue, and metastases occur relatively late. Its rate of growth in Japanese mice is relatively slow, so that the average weight at the end of five weeks is not over one gram.

The most striking peculiarity of this tumor is that it has been grown successfully in the first generation of the hybrids, obtained by cross-breeding Japanese and common mice, as well as in Japanese waltzing mice; while, on the other hand, all other mice inoculated, including hybrids of the second generation (*i.e.*, the offspring of hybrids of the first generation bred together), have constantly proved resistant to it.

In Japanese waltzing mice ninety-eight per cent of the inoculations have been successful. For the purposes of this experiment, therefore, series of Japanese waltzing mice and hybrids of the second generation were inoculated for comparative study.

The technic employed in the inoculations is as follows: A well developed tumor in a Japanese waltzing mouse is removed under aseptic precautions (the animal having been killed by chloroform), and placed in a sterile Petri dish. Then a small bit from the periphery of the tumor, which shows by its translucency that it is not necrotic, is taken and finely divided with a pair of fine scissors, and is then drawn up into the end of a small trochar. All instruments used are previously sterilized by boiling, and the trochars are sterilized before each inoculation. The point of the trochar is then inserted under the skin of the mouse to be inoculated in the posterior portion of the back where the hair has already been clipped. It is then carried forward beneath the skin of the right side to the region of the axilla, the contents are expelled by pushing home the plunger, the instrument is withdrawn, and the tissues around the site of the inoculation are then gently rubbed together a few times between thumb and finger. In this way a large number of mice may be inoculated from the same tumor.

In the first experiment a series of twelve young Japanese waltzing mice and twelve young hybrids of the second generation were inoculated, by the method just described, with small portions of a "Japanese" tumor of six weeks' growth. One Japanese waltzing mouse and one hybrid of the second generation were killed at the following periods after inoculation: twenty, thirty, forty-eight hours, three, four, five, seven, ten, fifteen, twenty, and twenty-eight days. The skin of the right side was in each case carefully dissected back and the tumor material exposed. This was inspected, removed by cutting off the skin flap, and placed immediately in Zenker's fluid.

Later a second series of mice was inoculated from another

"Japanese" tumor, slightly larger doses being injected. This consisted of six Japanese waltzing mice and twelve hybrids of the second generation. In this series one "Japanese" and two hybrids were killed at the end of each of the following periods: seven, eight, nine, ten, eleven, and twelve days.

The results are as follows: In both the Japanese mice and the hybrids the central portion of the introduced tumor becomes necrotic, but the cells at the periphery of the mass continue their growth, and mitotic division is frequent. The relative amount of necrotic tissue varies in different individuals. The introduced stroma, on the other hand, degenerates and becomes involved in the central mass of necrotic tissue, so that at the end of four days it is no longer distinguishable. The injected tumor material is surrounded by an inflammatory exudate which probably represents a reaction to the trauma of inoculation. This is sero-fibrinous or fibrino-purulent, and contains many large cells of endothelial origin which become phagocytic near the tumor mass. In some cases the tumor mass is to a large extent surrounded by a space containing serum and lined by a layer of fibrin. All the early stages — from twenty to forty-eight hours inclusive — show large numbers of polynuclear leucocytes and large endothelial phagocytic cells lying in the surrounding tissues, filling the space immediately around the tumor, and in many cases penetrating throughout the interstices of the tumor mass. After forty-eight hours, however, this exudate begins to be resorbed, and at the end of four days there remain in the surrounding tissues merely a trace of the fibrin and a few scattered inflammatory cells: polynuclear leucocytes, eosinophiles and large endothelial cells.

Meanwhile proliferative changes occur in the connective tissue of the host. In the twenty-four-hour specimens many of the connective tissue nuclei are large and vesicular, and have their chromatin gathered into one or two large masses. Proliferation of this tissue has actually begun at this stage as occasionally a cell may be found in mitosis. From this time on, the process continues until the tumor is surrounded by an area of very cellular connective tissue. Into this zone of fibroblasts the proliferating tumor cells begin to push their way, while at the same time the proliferating connective tissue cells invade, though apparently much more slowly, the interstices of the original tumor mass. This condition is seen in the specimens at the end of three days both in the case of the susceptible and of the non-susceptible animals.

At this time also (three days) the neighboring blood vessels have become much dilated, and their endothelium is swollen (mitotic division of endothelial cells may occasionally be found at this time). The next specimens, however (four days), show the beginning of capillary extension. Endothelial processes, some of which have acquired a lumen and contain red blood corpuscles, are found in the zone of young connective tissue and some of them have penetrated between the outgrowing masses of tumor cells. This process continues, so that in the tumors of five days' growth the proliferating connective tissue and blood vessels form a new stroma for the rapidly proliferating tumor epithelium. The same is true of the seven day tumors, of which the one from the Japanese mouse shows the more necrosis, but has acquired its new stroma, and is very rapidly growing in some portions. The specimen from the hybrid (seven days) is a well established and vascularized tumor — a perfect reproduction of the parent growth.

This series shows, therefore, that the tumor by acquiring a new stroma from the tissues of the host becomes established in both susceptible and non-susceptible mice. The process is similar to that described by Bashford and Murray[2] as occurring in the Jensen tumor when inoculated into susceptible animals. Definite evidence of amitotic division of connective tissue as described by Bashford and Murray[2] was not found, although certain appearances were suggestive of it. Its main points may be summarized as follows:

(1.) A continuous growth of those tumor cells which are situated near the periphery of the injected mass and are therefore in a position to receive nutriment; and a necrosis of the cells in the central portion of the mass furthest from the supply of nutrition.

(2.) A reaction to trauma—an inflammatory exudate varying in amount, which is for the most part resorbed by the fourth day.

(3.) A proliferation of the surrounding connective tissue forming a zone or layer of fibroblasts about the tumor, which has begun twenty-four hours after inoculation.

(4.) An extension of the neighboring capillaries into this zone.

(5.) An extension of the tumor into the very cellular and vascular capsule thus formed and a concomitant penetration of the original area of the tumor mass by the proliferating connective tissue and growing blood vessels. In this way the tumor epithelium acquires a new stroma.

After it had thus been shown that the "Japanese" tumor becomes well established and begins vigorous growth in mice known to be resistant to it, it remained to trace the fate of these small tumor nodules, and determine if possible the factors which bring about their ultimate destruction. The study of the remainder of the first series of implants (*i.e.*, those taken after ten, fifteen, twenty, and twenty-eight days' growth) showed that while in the Japanese mice the tumor had continued its growth, in the hybrids it had in every case undergone necrosis. The ten-day specimen showed a distinct nodule made up of necrotic cells and surrounded by a layer or zone of connective tissue and cellular exudate, but no living tumor tissue was present. At fifteen days this mass had been partially absorbed, at twenty days it was barely visible, and at twenty-eight no trace of it could be found. In view of these facts the second series (twelve hybrids of the second generation and six Japanese waltzing mice) were inoculated and taken for study after the following periods of growth: Japanese, seven, eight, nine, ten, eleven, and twelve days; hybrids, seven, eight, nine, ten, eleven, twelve, and fourteen days.

The histological study showed that in all the Japanese mice in this series the tumor was continuing its growth. Processes of tumor cells were seen in every case to be pushing their way into the zone of growing connective tissue about the tumor.

Many of the specimens, however, showed a relatively large amount of central necrosis. Probably this was due to the use of slightly larger doses of tumor material in this series, with the result that in each case there was a larger mass of tumor tissue which was deprived of nutrition after implantation. Notwithstanding these large areas of necrosis, in none of them was there to be noted any appreciable inflammatory reaction on the part of the surrounding tissues. All the nodules had received a new stroma from the host, and in every case the blood supply of the actively growing portions of the nodules was seen to be intact. One of these tumors (eleven-day specimen) was only very slightly larger than the mass originally injected, but most of them had at least doubled their original size. The twelve-day specimen had reached the size of a small pea.

Examination of the tumors from the hybrids, on the other hand, disclosed the following conditions. — In most of the cases (taken after seven days) the tumor tissue was reduced to a thin layer of cells (some of which were still dividing) surrounding a relatively large area of necrosis. One tumor, however (a ten day specimen), was growing vigorously, and had reached at least three times the diameter of the original injection, but was infiltrated with leucocytes. Both specimens of seven days' growth and one of nine days showed a fairly large amount of living tumor tissue. On the other hand, in three hybrids killed ten, eleven, and fourteen days after inoculation all trace of living parenchyma had disappeared. The whole series, then, apparently presents various degrees of the same process, *i.e.*, the destruction of the tumor.

A very important factor in this process seems to be the accumulation of cellular exudate in or about the tumor mass. This is seen throughout the series in case of the hybrids, and is not present in any of the tumors from Japanese mice. It consists of polynuclear neutrophiles, eosinophiles, lymphoid and plasma cells. In some cases one type of cell predominates, in others another. Immature leucocytes and cells resembling those in the germinative centers of lymph nodes are also to be seen in the exudate. Even the largest and most flourishing nodule in the series of resistant animals (the ten-day specimen mentioned above) is infiltrated throughout with lymphoid and plasma cells. The blood supply of this tumor has not yet been interfered with, but these cells are distributed throughout the entire growth. This tumor is exceptional in that it shows practically no necrosis.

Apparently following or associated with this cellular infiltration there is evident in most of the other specimens from the resistant mice an excessive production of collagen fibrils on the part of the connective tissue cells of the new-formed stroma (see Fig. 9). This begins about the edge of the central necrotic area. It results in a widening of the interstices of the tumor with a consequent crowding together of the tumor cells and thus an impairment of their nutrition. So we have two processes, a cell infiltration and an increased formation of connective tissue fibrils, each of which may be considered a part of a distinct inflammatory reaction, and either or both of which is present to a marked degree in every case in this series of non-susceptible mice.

The parenchyma of those tumors in which the process of degeneration is apparently well advanced shows no tendency whatever to invade the surrounding tissues. Although the presence of occasional mitoses shows that it is still in a state of proliferation, there are evident no new outgrowths of tumor epithelium. The explanation of this condition seems to lie in the fact that the nutrition and probably the blood supply of this tissue is insufficient. In no tumor where peripheral extension is practically at a standstill, and central necrosis has considerably advanced, is it possible to discover any blood vessels which have remained patent and are in close relation to the tumor cells.

The following table shows the nature and the degree of the inflammatory reaction and the condition of the tumors in each of the non-susceptible mice of this series.

TABLE I. SERIES II. HYBRIDS OF THE SECOND GENERATION.

Mouse Number.	Days of Growth.	Inflammatory Cellular Infiltration.	Reaction, Fibrous Change of Connective Tissue.	Condition of Tumor Tissue.
2459 A ..	7	+	—	Some growth peripherally. Mitoses relatively few. Necrosis relatively large.
2469 B ..	7	+	+	Mitoses few. Peripheral extension slight. Necrosis relatively large.
2461 A ..	8	+	+	Tumor tissue in a thin layer around large central necrosis. Mitoses very few. No peripheral extension.
2471 B ..	8	+ +	Slight.	Tumor tissue in a thin layer around large central necrosis. Mitoses very few. No peripheral extension.
2475 C ..	9	+	+ +	Actively growing. Large central necrotic mass.
2465 A ..	10	+	—	Well developed and actively growing. Very little central necrosis.
2477 C ..	10	+	+	Tumor tissue practically gone, a few faintly staining cells remaining. No mitoses.
2479 C ..	11	Slight.	+ +	Thin broken ring of tumor tissue seen in section around large necrotic area.
2473 B ..	11	Slight.	+ +	Tumor tissue gone.
2467 B ..	12	+ +	+	Thin layer of tumor tissue remaining. No peripheral extension.
2392 D ..	14	+	+ +	Thin layer of tumor tissue remaining. No peripheral extension.
2394 D ..	14	+	+ +	Tumor tissue gone.

(NOTE.—The mice of this series were from four different litters. Those from the same litter are designated by the same letter following their number in the first column.)

From the data at hand we are thus justified in concluding that the process of destruction of the tumor in the non-susceptible mice is somewhat as follows: After a period of growth on the part of the tumor, the duration of which varies with different individuals, there appears in the surrounding tissues an aggregation of exudative cells. It is probable that this reaction may take place in some cases before the tumor has become well established and as a continuation of the original reaction to trauma. On the other hand, there is in most cases a vigorous growth on the part of the tumor for from seven to ten days before the appearance of this second cellular exudate about the tumor. The cells of this exudate lie in the neighboring areolar tissue, and infiltrate the zone of connective tissue immediately about the tumor. Apparently as a result of this process the nutrition of the latter suffers. The cells furthest from the periphery degenerate, and thus the area of centrally situated necrotic tissue becomes increased. Later the exudate in many cases actually separates the tumor cells from the connective tissue of the host. This destruction of the relation of tumor cells to host's tissue apparently renders the former unable to continue their growth. Associated with this impairment of nutrition there occurs the previously mentioned overproduction of collagen fibrils on the part of the connective tissue of the stroma. This takes place at the edge of the central necrosis, separates the tumor cells of this region into thin strands, and still further impairs their nutrition. Thus the area of central necrosis enlarges.

At the same time peripheral extension of the tumor becomes diminished. This seems to be due to a general impairment of the nutrition of the whole nodule, caused by the factors just mentioned. Not only may the exudate which collects in the region of the tumor separate it from its capsule, and thus deprive it of its nutrition, but the entire nodule may eventually become infiltrated also with exudate. Thus the tumor cells become reduced to a thin layer surrounding a large necrotic mass. As the cells of this layer are still subject to the same conditions which have brought about the degeneration of the rest of the nodule, the process continues. The tumor epithelium no longer sends outgrowths into the surrounding connective tissue, although the presence of a few mitoses shows that growth is not entirely at a standstill. They stain rather more faintly than the cells of a healthy tumor, and are not so closely packed together. Finally they all become completely degenerated, and there is left simply a mass of necrotic material. This sometimes contains a number of leucocytes, and usually shows large areas of very fibrous connective tissue, the result of the changes in the new stroma which have been mentioned above. Surrounding this is a zone of fairly dense connective tissue and around this there is more or less cellular exudate. Finally this exudate and also the necrotic material become absorbed.

With regard to theoretical considerations it is evident that the facts here presented neither support nor tend to disprove the hypothesis of Ehrlich[7] in which he advanced the idea that in resistant animals the failure of the growth of the tumor is due to the lack of some specific substance ("X substance") which stimulates the tumor cells to proliferate. In these experiments there is no evidence as to the presence or absence of such a substance. They show, on the other hand, a reaction on the part of the host which consists of a more or less mechanical interference with the nutrition of the tumor. The failure of the tumor to grow in insusceptible mice is in this way satisfactorily accounted for without considering the possible presence of a hypothetical "X substance."

The experiments of Russell lead him to consider that in mice naturally immune or artificially rendered so by injections (as described above) the tumor degenerates because it receives no stroma. In his opinion either the tissues of the host fail to react to the stimulus of the newly implanted cancer cells or these cells fail to exert their usual influence. He concludes, however, that it is probable that the latter condition obtains, *i.e.*, the absence of a typical "fibroblastic and angioplastic stroma reaction" is due to suppression of the chemiotactic properties of the tumor cells. In the case of the tumor inoculated into animals of a different species (*e.g.*, rats), on the other hand, the sudden disappearance of healthy growth after some days is due, he believes, to the formation of specific cytolysins or cytotoxins in the body of the host.

In the present experiments it must be remembered that we are dealing with animals of very closely related varieties capable of interbreeding freely; and that the non-susceptibility of one of these varieties is as absolute as is that of a different species. It is clear that in this case there is no failure of stroma reaction and that immunity in the resistant animals is developed only after several days of vigorous growth on the part of the tumor. In fact the stroma reaction eventually surpasses that of susceptible mice. The destruction of the growth which then ensues, however, is apparently dependent upon an interference with its supply of nutrition. It has been suggested that the inflammatory changes observed may be secondary to a specific serum reaction which reduces the growing nodule to the status of misplaced normal epithelium, and that the process is then similar to the retrograde changes seen in the destruction of such tissue. While the possibility of such a condition cannot be denied, the fact that the necrosis begins at the centers of the nodules, progresses slowly toward the periphery, and is always associated with a definite interference with nutrition, renders it more probable that the immediate cause of the degeneration of the tumor is the appearance of the inflammatory reaction in and around the growth, which cuts off its nutrition. In rats, on the other hand, Russell[8] observed that the necrosis was at first more pronounced, if anything, at the periphery of the nodule and was thus due in all probability to the presence of specific substances in the serum of the rat. In the present instance we have, at all events, a specific immunity developed in a variety of mice very closely related to that in which the tumor originated and grows. Whether or not this immunity is fundamentally of the same nature as that developed in animals of a different species, it is at present impossible to state with certainty. It is at any rate a definite immunity, which develops after an interval of at least seven days from the time of inoculation, and manifests itself in the appearance of an inflammatory reaction which mechanically interferes with the nutrition of the tumor.

SUMMARY.

In susceptible Japanese waltzing mice inoculated with the "Japanese" tumor, the tumor receives a new fibrous and vascular stroma from the tissues of the host.

In the non-susceptible hybrids of the second generation (the offspring of hybrids obtained by mating Japanese and common mice) the tumor receives a new stroma and blood supply in the same manner as in Japanese mice.

After a short period of active growth (usually about a week) the tumor in the non-susceptible mice is surrounded by an inflammatory exudate which impairs its nutrition.

Apparently as a part of this inflammatory reaction, in many of the non-susceptible mice there occurs an overproduction of fibrils on the part of the more centrally located portions of the new stroma of the tumor.

No such inflammatory reaction is seen in case of the tumors of a corresponding age in Japanese mice.

Probably as a result of impairment of nutrition, due to the factors already mentioned, peripheral extension of the nodule (in the resistant mice) ceases, central necrosis at the same time advances, and ultimately all the tumor tissue undergoes necrosis and absorption.

In the non-susceptible mice there is developed, therefore, an active immunity the mechanism of which is apparently an inflammatory reaction which interferes with the nutrition of the tumor.

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DESCRIPTION OF PLATES.

PLATE LVI.

FIG. 1. — Tumor in Japanese mouse after twenty-four hours' growth. Reaction to trauma. Tumor mass surrounded by a space containing serum and lined by a layer of fibrin.

FIG. 2. — Tumor in Japanese mouse five days after implantation. Formation of new stroma. Outgrowth of tumor cells. Ingrowth of connective tissue and blood vessels (compare with Fig. 3).

FIG. 3. — Tumor in hybrid of second generation five days after implantation. Formation of new stroma (condition same as that shown in Fig. 2).

FIG. 4. — Tumor in Japanese mouse after seven days. Tumor well established. It has acquired its new stroma and is invading the surrounding tissues (compare with Fig. 5).

PLATE LVII.

FIG. 5. — Tumor in hybrid of second generation after seven days. Condition of tumor similar to that shown in Fig. 4. Note, however, the beginning cellular infiltration of the tissues surrounding the tumor.

FIG. 6. — Tumor in Japanese mouse after nine days. Tumor well established and actively growing. Note absence of inflammatory reaction (compare with Figs. 7 and 5).

FIG. 7. — Tumor in hybrid of second generation after nine days. Tumor well established and actively growing. Note marked inflammatory reaction about tumor.

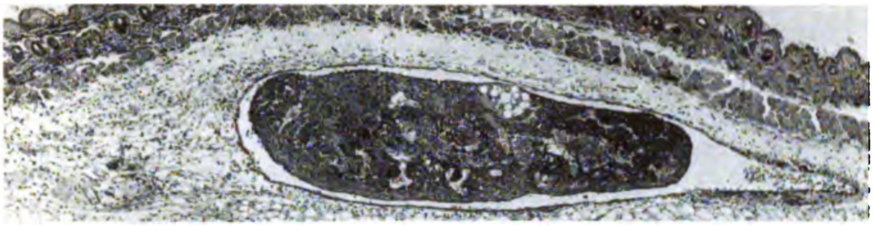
PLATE LVIII.

FIG. 8. — Tumor in Japanese mouse after ten days. Tumor especially well developed.

FIG. 9. — Tumor in hybrid of the second generation after eleven days. Mallory's connective tissue stain. Note the darkly stained areas of connective tissue fibrils. The living parenchyma of this tumor is reduced to a narrow layer surrounding the relatively large central area which is necrotic and which is darkly stained in this specimen. The parenchyma

is not well shown, but is for the most part separated into thin strands by the masses of fibrils formed by the connective tissue of the new stroma. Moderate infiltration of surrounding tissues.

FIG. 10. — Tumor in hybrid of the second generation after twelve days. The inflammatory reaction in this case has taken the form of a marked cellular infiltration. Peripheral extension of the tumor has ceased, central necrosis has advanced, and the living tumor cells are reduced to a relatively thin layer. The tumor epithelium is to a large extent cut off from surrounding connective tissue.



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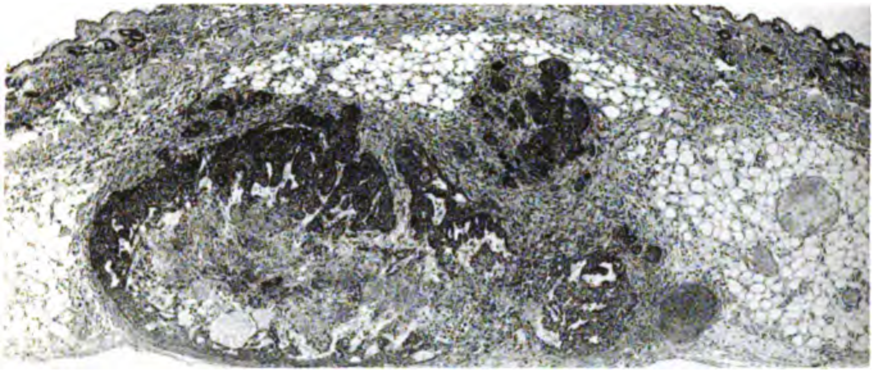

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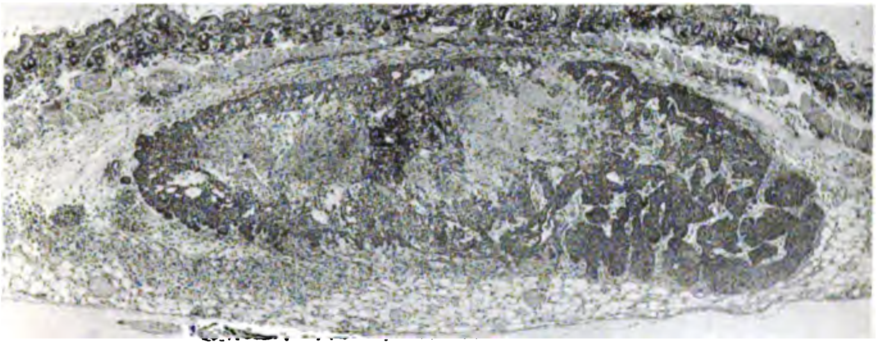

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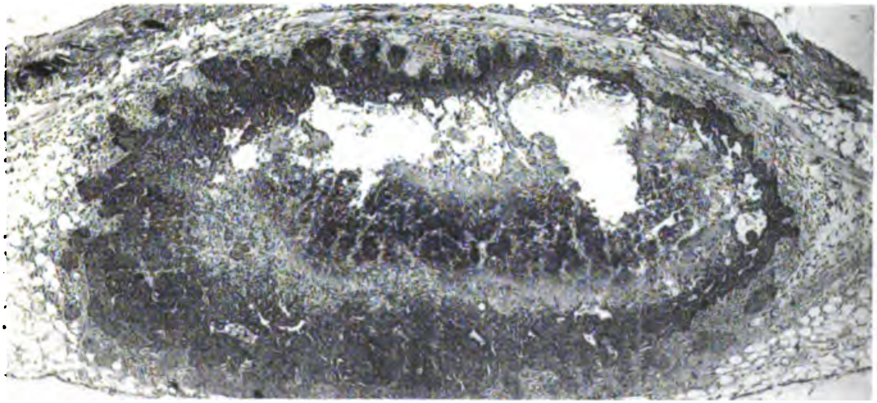

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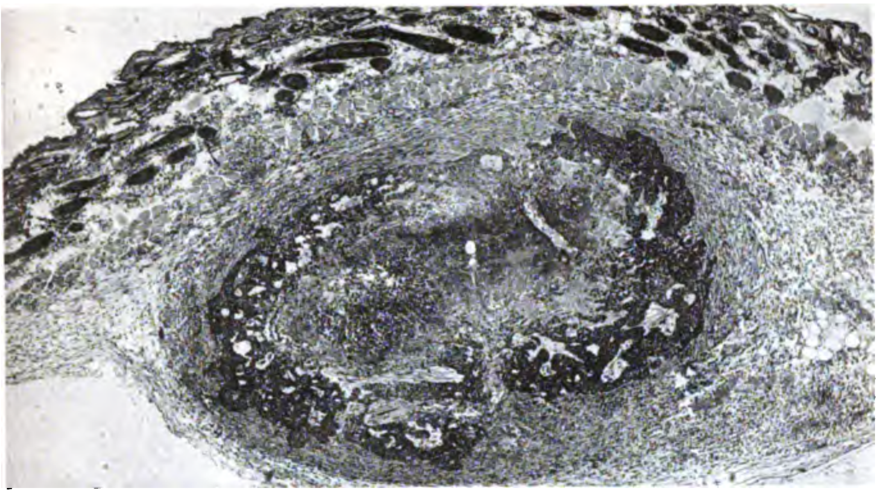

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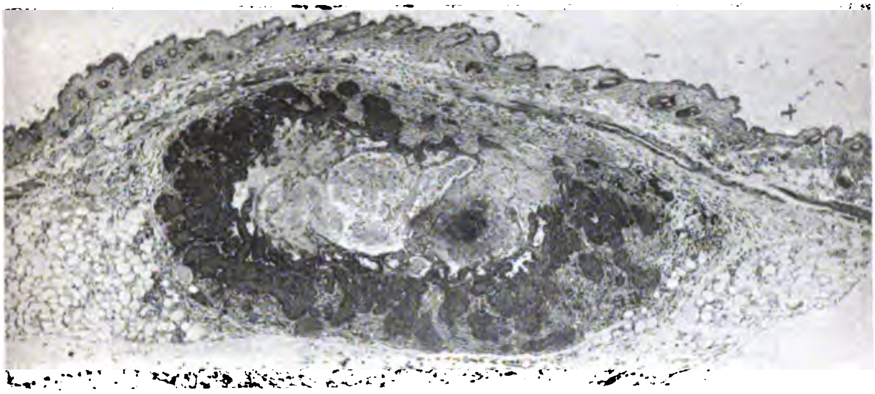

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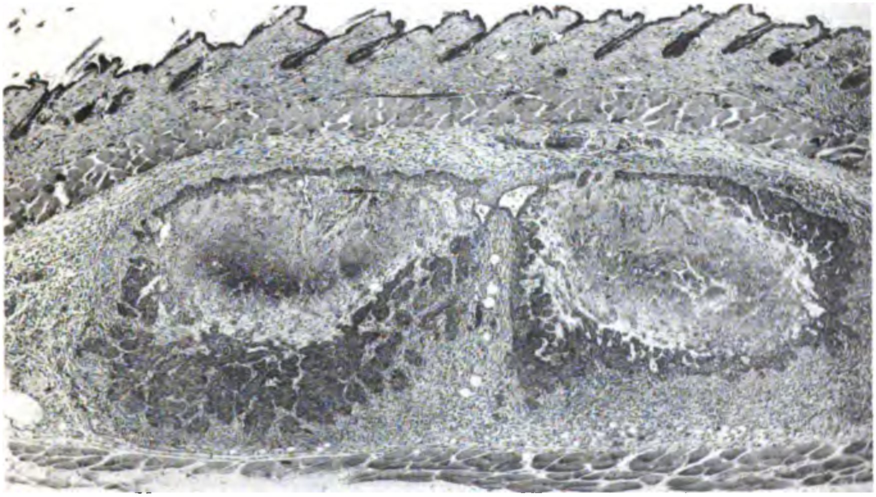

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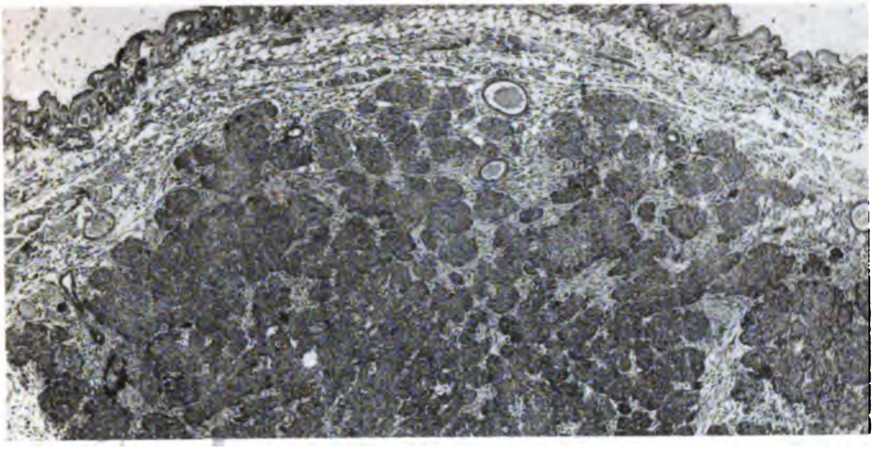
Inoculable tumor.



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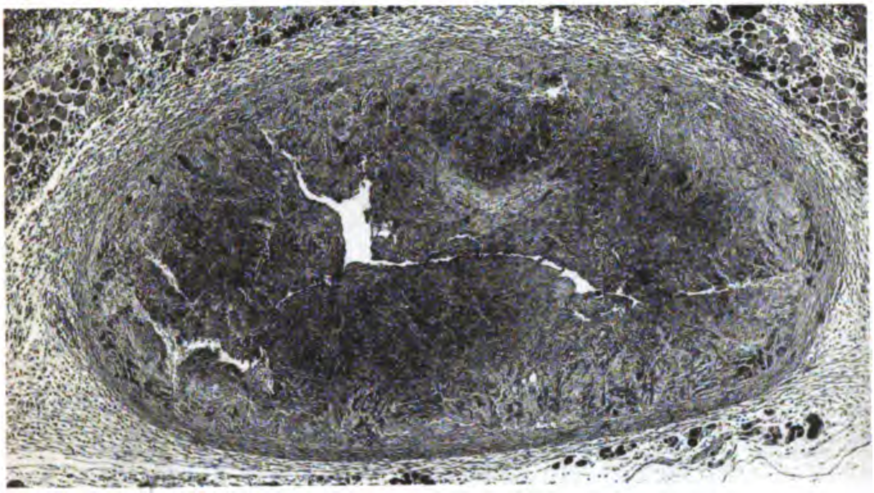

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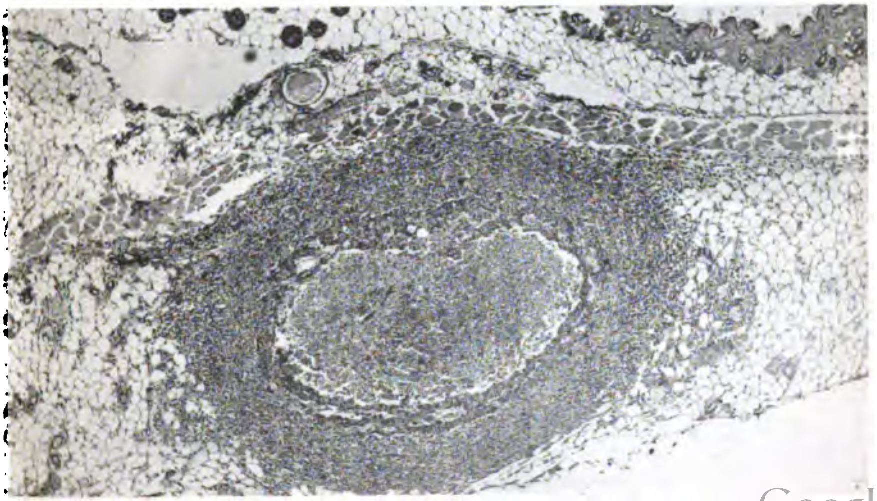

10

THE EFFECT OF TRYPSIN ON CANCER AND ON THE GERM CELLS IN MICE.*

STEPHEN RUSHMORE, M.D.

In January, 1906, there appeared in the British Medical Journal[1] an article under the caption, "The Effect of Trypsin on the Living Cells of Jensen's Mouse Tumor." It was from the pen of Mr. Beard, Lecturer on Embryology at Edinburgh University. In this article is the statement that the action of trypsin upon the cancer cell "is to pull down the cancer albumen" — "to cause cancer to degenerate." In a previous article[2] Beard states that for the treatment of cancer, in trypsin, "the physician has had forged for him a light and not dangerous weapon, only second, if not equal, in potency to the surgeon's knife." These and similar statements have attracted considerable attention, and the question has arisen, "Will trypsin cure cancer?"

Numerous investigators have put the question to the test of experiment in the treatment of human patients. The reports as to the efficacy of this new therapeutic agent are conflicting, or at least are not in agreement, and in one case there was actual conflict of opinion as to whether the patient was cured or not.[3, 4, 5]

The question would seem to be easy of access by experiment on animals, and Beard himself applied this test. His experiments are not conclusive, however. He employed three mice with tumors. Two were treated with injections of trypsin, the third was kept as a control. In about ten days one of the animals under treatment died from some unknown cause. In about three weeks the control died, with a tumor as big as the "terminal phalanx of a man's thumb." The second mouse under treatment, the tumor of which was then the size of a lentil, was killed, and the tumor examined under the microscope. Of this Beard says: "The tumor was in advanced degeneration, shrinking away to

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nothingness, and quite harmless. It appeared probable to us that, at the time we killed it, its 'cure' from cancer was not far distant, and the microscopical examination confirmed this opinion. Even without further treatment, the tumor would in all probability have been absorbed shortly, or its remains cast out." This is the experimental evidence for the hope that trypsin will cure cancer.

At the suggestion, and at first under the direction of Dr. Wm. P. Graves of Boston, and with the cordial assistance of Dr. E. E. Tyzzer, a further experimental study was undertaken.

The scope of the experiments may be briefly outlined as follows:

To determine the effect of trypsin on the growth of tumors in various stages of advancement.

(1.) Very large tumors.

(2.) Moderately large tumors, but apparently definitely advanced beyond the stage where spontaneous regression might take place.

(3.) Early tumors, at a time when spontaneous regression might occur.

(4.) In a fourth experiment, the effect of relatively large amounts of trypsin in normal animals was noted.

(5.) Then the effect on conception and pregnancy of the injection of non-fatal doses of trypsin.

In addition, histological changes were studied in three groups of cases:

(6.) Tumors into which or near which trypsin had been injected.

(7.) Sexual glands of normal adult animals, into the general circulation of which trypsin had been introduced.

(8.) Sexual glands of the male guinea-pig into which trypsin had been directly injected. For the material of this last experiment I am indebted to Dr. Graves. In all the other experiments mice were used, for which I am indebted to Dr. Tyzzer. The tumor was an adenocarcinoma — the Ehrlich tumor. The trypsin employed was a stock preparation put up in sealed glass ampoules, containing about twenty minims in each. By the manufacturers it was stated that it is not possible to make a quantitative determination of the amount of the trypsin; the determination of the strength is made by means of the digestive power of a given quantity of the solution. As, therefore, accuracy is not possible, the stock preparation was arbitrarily called one hundred per cent trypsin for the sake of convenience. This, however, contains about sixty per cent glycerine. The quantity used varies in different experiments and is there stated. In human patients doses larger than one ampoule, or twenty minims, have been used with safety. Regarding seventy kilograms as the average weight of a patient, and twenty minims as an average dose, it is evident at once what enormous amounts of trypsin were used; approximately one-fiftieth of the dose for a human patient was employed for a mouse. The injections were made at intervals of two to three days.

(1.) In the first experiment three mice were taken; the tumors were very large, evidently in a late stage; the general condition of the mice was good. Injections of fifty to one hundred per cent solutions, two to four minims, were made directly into the tumors, with slight decrease in size, which might easily be explained by the sloughing of the tumor. The mice died in from six to nineteen days after the beginning of the treatment with practically no appreciable local improvement. The tumors were measured before each injection.

(2.) In the second experiment, two groups of mice were taken. The first consisted of old mice with tumors well advanced — slowly growing — no sign of sloughing. In one mouse there was noticeable improvement: in about four weeks the tumor had disappeared. There remained a questionable trace in the form of a very small crust at a point where there had occurred slight sloughing. The treatment was stopped, but ten days later the tumor was again palpable, and caused the death of the animal, in spite of treatment, in about one month. The other mice in this experiment, including the group of younger mice in which the tumors grew more rapidly, died after varying intervals, from the growth of the tumor, while under treatment. Two to ten minims of from two to ten per cent solution were injected.

(3.) In the third experiment ten young mice of a susceptible strain were inoculated with cancer tissue. Before the treatment with trypsin was begun, two of these died, of which one showed no sign of tumor; the other showed a small growth. Of the remaining mice, one showed no tumor at any time. Treatment was begun in seven on the fifteenth day after inoculation, at a time when the tumors might spontaneously disappear. They varied from the size of a split pea to one centimeter in diameter. The treatment consisted of three injections a week for nine weeks into the subcutaneous tissue, at a distance from the tumor. For two injections trypsin was used; for the third, amylopsin. (This use of amylopsin also is recommended by Beard.) In three mice the tumor disappeared in about twelve days. Treatment was stopped for a week, then resumed. There was no reappearance of the tumor. In four the tumor progressed much as in the mice not under treatment, ultimately causing death. This gives a percentage of cure or spontaneous recovery, according to the point of view, of forty-three, a little higher than the percentage usually given for spontaneous recovery. But since the different strains of mice vary and the number is so small, this slight difference of from ten to fifteen per cent may not be attributed to the effect of the trypsin. Four minims of ten per cent solution of trypsin or amylopsin were used at each injection.

(4.) In the fourth experiment, large amounts of trypsin were injected into three normal mice. All became toxic after a few days of treatment, and two died; the third recovered after stopping the treatment. The tissue from these animals was not studied under the microscope.

(5.) In the fifth experiment there was one mouse belonging to the group in Experiment III. in which a tumor disappeared. While under treatment this animal became pregnant and gave birth to normal young. At autopsy there was no trace of the tumor, which had been present at an early stage. The case is interesting on account of the claims made by Beard as to the relation of cancer to germ cells, and the specificity of the action of trypsin on both kinds of cells. Functionally, at least, there was no interference with the action of the germ cells in the ovary.

(6.) The study of histological changes covers three experiments. From one to four injections were made into or near small tumors, which were removed later at operation or autopsy, and fixed in Zenker's fluid. If trypsin has a deleterious effect on the tumor cells, we should expect to find it extending directly from the point of injection, or by the blood stream. In the tumors examined, which showed the usual central necrosis of even early tumors, the cells around the blood vessels were in the best condition, and there could be made out no especial injury of cells lying on the periphery of the tumor nearest the point of injection.

(7.) In the seventh experiment six normal young adult mice were taken — three males and three females. They were treated with trypsin solution for three weeks, three injections a week. They were then killed and the tissues fixed in Zenker's fluid. Under the microscope the sexual glands cannot be distinguished from those of normal animals.

(8.) In the eighth experiment, trypsin was injected directly into the testicle of guinea-pigs, and the tissue removed at autopsy forty-eight hours later. As the trypsin is prepared with glycerine, about sixty per cent, glycerine alone was injected as a control. Microscopical examination showed focal necrosis of the injected area, the epithelial cells being more completely destroyed than the connective tissue. The destruction was slightly more marked with the trypsin than with the glycerine alone. There could be made out no difference in the character of the effect of the trypsin and glycerine, and the glycerine alone.

These uniformly negative results suggest the question as to the presence of any active enzyme in the solution employed. Test-tube experiments were conducted with the amylopsin as well as with trypsin, employing fibrin from horse's blood.

A rather active proteolytic enzyme was present in each, the two solutions being apparently of approximately the same digestive power for fibrin.

In these experiments the number of animals studied is small, but the results did not seem to justify further investigation along just this line.

CONCLUSIONS.

The conclusions may be stated briefly. In these experiments trypsin had no appreciable effect in causing the cancer to disappear or in inhibiting the growth and function of the germ cells.

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A TRANSMISSIBLE CANCER OF THE RAT CONSIDERED FROM THE STANDPOINT OF IMMUNITY.*

FREDERICK P. GAY.

The occurrence in mice and rats of conditions of apparent immunity or resistance to transmissible tumors has attracted due attention from the beginning of the modern experimental investigation of cancer. The lack of definiteness of many results bearing on this aspect of cancer study is owing not so much to lack of consideration as to the inherent difficulty of the problem. The difficulty is due to the multiplicity of known or unknown conditions that modify any given experiment.

This study makes no claim to completeness in any given part, the confused and often apparently irreconcilable results of other observers, having made a somewhat skirmishing method of attack for the purpose of orientation, seem most serviceable. The reply of many of the experiments is negative or doubtful, and some of them undertaken to answer apparently the most logical inquiry are so problematical in result that they will not even be mentioned. As a result of the work has come the knowledge of certain conditions that are controllable, as well as distinctly promising data that merit more careful study.

In order to simplify experimental conditions as far as possible most of my work has been done with a single strain of tumor, a tumor of the rat already described by Flexner and Jobling[1] to whom I am indebted for a transplant. This tumor originated in the seminal vesicle of a white rat and, as found, was described by Flexner and Jobling as a mixed cell sarcoma; they have since noted its transformation after several generations into a "malignant adenoma" or carcinoma. Transformations have been described in mouse tumors by Apolant, Ehrlich and Haaland,[2] and by Loeb[3] (Carcinoma

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transformed into Sarcoma) and in a rat tumor by Lewin[4] (Carcinoma to Sarcoma in a few generations). The Flexner-Jobling tumor as it came into my hands may be described as an adeno-carcinoma containing a considerable amount of connective tissue; it has not changed appreciably in histological structure during the eight generations through which I have cultivated it.

Reference will be made to a few incidental experiments that have been made with the Ehrlich and the Jensen tumors of mice. The rat tumor has been most serviceable on account of the larger size of the host (which permits the collection of an appreciable amount of blood), the rapidity of the growth of the tumor, and the frequently and relatively rapid occurrence of metastases.

The rats have been inoculated by means of a trochar needle inserted subcutaneously along the abdominal surface and pushed cephalad as far as the axilla, at which point the "load" of the needle is expelled. It has been emphasized by Bashford, Murray and Cramer,[5] and by Bridré[6] that inoculation at this point results in the largest percentage of takes, probably owing to the vascularity of the region. The tumors used for transplantation have purposely been of considerable size, say from one and one-half to two centimeters in diameter; it has been noted by Jensen[7] that small tumors are less effective for implantation than older ones, probably owing to the fact that actively mitotic cells are less resistant. Tumors of this size correspond to a growth of from four to six weeks and contain a large soft center of broken down detritus surrounded by a more or less considerable shell of actively growing tissue. It has further been pointed out by Clowes and Baeslack[8] that such broken down tumors are characteristically more "virulent," which fact they correlate with an increase of calcium and absence of potassium. Tumors that have grown to this stage tend to become infected through skin abrasions and become sloughing and foul smelling, although the living periphery apparently continues to grow with undiminished vigor; such infected tumors have been avoided in transplantation. After killing the rat with chloroform, the tumor to be transplanted is removed with aseptic precautions and the growing shell of tumor removed with sterile scissors and cut into small pieces. The importance on the result of the amount of tumor used for inoculation has been recently emphasized by Bridré, and by Gierke,[24] who consider that many apparent discrepancies between results obtained by different observers may be entirely attributable to this factor. Without employing an emulsion of cells, which from the observation of others seems less suitable for the transplanting, or without weighing the small bit of tissue injected, which seems a well nigh impossible refinement, there has appeared to be no way of obtaining more than approximate uniformity of dosage. An amount of living tissue equal to approximately half a match head in volume has been used for each animal. The variations in amount incident to this method are not, in my judgment, sufficient to render my own results other than comparable and are certainly not so marked as the variations between different observers.

In all later experiments young rats weighing from fifty to seventy-five grams have been employed as they are admittedly more susceptible than adult animals (Bashford, Murray and Cramer, Lewin and others). The source of supply, or race of white rats used, has seemed to be of very distinct importance in reference to the percentage of successful inoculations. Such racial differences of susceptibility have been particularly emphasized in the case of mouse tumors by Haaland,[9] who found that a simple change of locality was often sufficient to render individual races either more or less susceptible. Lewin has, however, noted no such differences in racial susceptibility to his rat tumor. Jobling (personal communication) has found no differences in the percentage of "takes" of his tumor in rats from different dealers. With such definite information as regards rat tumors I was not prepared to meet with the marked variations in racial susceptibility which have been evident in my hands. Many experiments have been rendered inconclusive through a failure to appreciate this factor from the beginning. During the last few months it has unfortunately been impossible to obtain animals of the most susceptible strain. In the following table is summarized my findings in this respect. The animals included are only such as have received no treatment designed to influence the growth of the tumor. Not all the strains of rats were given any individual tumor, as would be desirable if an experiment for determining this racial variation alone had been planned. It is obvious that the "virulence" of the tumor may vary from generation to generation, and that no given tumor is strictly comparable with any other. In order to allow for this variable factor the number of different tumors used in implanting each given race of rats is included in the following summary:

TABLE I.

Percentage of positive inoculations in rats obtained from different sources with the Flexner-Jobling tumor.

Source.	No. of Different Tumors.	Total of Rats.	Positive.	Negative.
A. Adult rats (100 grams +):				
C.	7	25	25	0
P.	2	8	1	7
W.	1	2	0	2
B. Young rats (50–75 grams):				
P.	2	20	2	18
W.	4	8	2	6
H.	1	3	0	3
L.	8	140	70	70

It will be seen that whereas not a single rat from source C has failed to take a tumor that the next best source, L, took in only fifty per cent of the cases and in the animals of source P only three out of twenty-eight gave positive transplants.

Visible metastases; as has been noted by Flexner and Jobling, occur regularly, following successful transplants of the tumor. These authors by means of transplants into several regions obtained metastases in the lungs, peribronchial nodes, and kidneys. Using the subcutaneous axillary transplantation, I have obtained metastases in the lungs and peribronchial lymph nodes usually, and in the adjacent axillary lymph nodes only rarely. The metastases of the lungs may be so extensive as to invade and replace an entire organ. The time of their occurrence after inoculation would seem to be of considerable importance, as will be found in a later consideration of re-implantation of tumor-bearing animals at successive periods.

In considering the time after implantation at which metastases occur, there is evidence that rats of the most susceptible strain produce metastases earlier than do those of a more refractory strain. The fact that different individual tumors and also successive generations of the original tumor were employed with presumable differences in infectivity, render this comparison of the metastasis formation not strictly exact. It was noted that during six generations of the tumors and as a result of implantations from seven different tumors, one hundred per cent (*i.e.*, twenty-five) of the adult rats from dealer " C " showed tumors. The metastasis formation in such of these animals as were used as controls and not subjected to influences that might affect tumor growth, was as follows:

In three animals killed on the twenty-fifth, thirty-fourth, and thirty-seventh day after implantation no metastases were found. In eleven animals killed from the thirty-seventh day (3) to the sixty-eighth day metastases were found in all.

The young rats from source " L " implanted from eight different tumors took the tumor in fifty per cent (seventy out of one hundred and forty).

In this less susceptible strain, metastasis formation would seem to occur later and with greater irregularity than in the " C " rats, thus:

Of six tumor rats killed on the fifty-eighth to sixtieth day metastases were present in four, and of twelve animals killed from the ninetieth to the one hundred and fifty-sixth day, metastases were present in six only.

This summary, then, would seem to show that in rats moderately susceptible to the tumor, metastasis formation is more delayed even in those animals in which the tumor has "taken."

The metastatic nodules in the lungs resemble the original growth histologically, and I have tried to determine whether such metastases would prove equally or perhaps more virulent than the original tumor. A rapid transformation of the tumor in a few generations back to its original sarcomatous form was also a possibility. In the following generations rats from the same highly susceptible source (C) were used throughout:

I. — Rat F. B. 5, bearing a tumor that had been growing five weeks was chloroformed and the tumor used to inoculate.

II. — Rat F. C. 4, which was killed on the fifty-fifth day and showed many metastases in lungs and about heart.

III. — Rat F. D. 15 was implanted with lung metastasis from F. C. 4 on right side and with bits of original tumor from the same animal on the left side. The tumors grew equally well on both sides. Animal killed on thirty-seventh day showed many metastases in both lungs.

IV. — Rat F. E. 9 was given metastasis from F. D. 15 subcutaneously, and Rats F. E. 2 and F. E. 7 (among others) were given the original non-metastatic tumor from F. D. 15. On the thirty-seventh day all three were killed. The tumors in F. E. 2 and F. E. 7 measured 2.6 x 2.2 and 2.2 x 1.9 centimeters, respectively, and in F. E. 9, 4.25 x 3.5. F. E. 2 showed two small metastases on lungs. F. E. 7, no metastases. In F. E. 9 both lungs were filled with metastatic nodules. The original tumors F. E. 2 and F. E. 7 had both suffered necrosis through the skin. The tumor in F. E. 9 although larger had not broken through, and was firmer in the center. Histologically F. E. 9 differed markedly from the other two, being much more cellular and containing less connective tissue.

V. — Rat F. F. 46 was given lung metastasis from F. E. 9. The tumor grew slowly and remained firm. On the sixty-first day it measured 4 x 3 centimeters, and the animal was chloroformed. The tumor had a firm necrotic center. The pleura of both sides and both lungs with the exception of the right lower lobe were entirely replaced by metastases. This tumor also was found histologically to contain more epithelial cells and less connective tissue than the ordinary tumor.

The impossibility of obtaining any more rats from this susceptible source for several months has unfortunately enforced the abandonment of experiments along this line. The metastatic tumor showed no greater infectivity in normally resistant rats than the original tumor.

There was no histological variation of cell picture other than the relative variations noted found in the original tumors nor in the metastases during these five generations. There would appear to be a distinct increase of virulence in the metastatic tumor as noted under IV. (*i.e.*, second generation of successive implantation from metastases compared with corresponding generation from original tumor). The tendency of the "metastatic" tumor to remain firm and uninfected, irrespective of tumor or size, seemed characteristic (IV. and V.), and differs from the picture of the non-metastatic tumor which is usually sloughing at this time. The relative increase of the epithelial elements in the last generations has already been mentioned.

The fact that transplantations from metastases "take" at least equally as well as transplants from the original tumors has so far as I am aware not been definitely stated by any one save Murray.[25] The entire question merits more attentive consideration.

In his first report on mouse tumors Jensen attributed the failure of fifty per cent of mice to take his tumor to a natural immunity. Clowes and Baeslack[10] noted that mice that have spontaneously recovered from the Jensen tumor are immune to subsequent implantation with the same tumor. That such an immunity is at least relatively perfect is now generally admitted. In the experience of Bashford, Murray and Cramer the immunity following the resorption of the first tumor is absolute for the same tumor and partial for other tumors. Bridré regards this immunity as only relative, for on second inoculation some of his animals took; he found, however, that none that had resisted two inoculations took a third. Michaelis[11] also regards such immunity as distinct but relative. Sticker,[12] who worked with a sarcoma in dogs, found that the sixteen per cent that had recovered from a first implantation were absolutely immune to a second. The question of this condition of increased resistance is complicated somewhat by the normal resistance to first inoculation. To what extent can the failure of the first tumor to grow completely without retrogression be attributed to a normal immunity and to what extent to an active acquired immunity following the implantation of the tumor? Can we assert that no tumor which has grown beyond such and such dimensions will never retrograde? Of course when a large tumor has grown and then disappeared it may be assumed to have been resorbed, although even under these conditions Gierke[13] has described tumors which, although apparently entirely resorbed, began to grow again on the inoculation of a second tumor. It is further evident that without control animals any time limit as to when a tumor may be judged as positive must be arbitrary.

In order to increase to the maximum the resistance in certain animals for purposes to be later detailed I have reimplanted tumors in rats that failed to take the first tumor, at two successive periods (from ten days to a month apart in different lots). Of sixteen such animals only one ever developed a tumor and that one only on the third implantation. It is of interest to note that the single tumor that took on the third trial was a metastasis from "metastatic" tumor F. F. 46 (see page 180).

Ehrlich[14] found that if spontaneous hemorrhagic tumors, which grow in only a small percentage of animals, are implanted, instead of tumors of high virulence, that the animals so treated are immune to virulent tumors.* And they are immune not only to virulent tumors of a cellular type similar to the one used for immunization but also to tumors composed of other types of cells. In other words, the immunity acquired is not absolutely specific, and for this reason has been referred to by Ehrlich as a "pan-immunity." As is usual in all matters pertaining to this complex problem other experimenters have obtained only relatively similar or even directly opposite results. Bridré, Haaland, and Michaelis find that treatment with one tumor will not protect against a tumor of a different type. Bridré, however, obtained an almost absolute immunity against mouse tumors by several subcutaneous injections of macerated tumor. The apparently divergent results of various observers are wisely attributed by this writer to differences in individual technic; Ehrlich, for example, used much more tumor material for preventive implantation than did Bridré. It has been clearly shown by Michaelis and by Clowes[15] that immunity can be produced only by the living cancer cells and not by those killed with heat or chemicals, nor yet by the nucleoproteids derived from them. We may conclude, then, that an undoubted increase of resistance to tumor implantation may be produced in rats and mice by the previous implantation, with or without temporary growth, of the same type of tumor, and perhaps, under certain conditions, of other tumors.

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* It has recently been shown by Murray that these tumors will transplant if smaller amounts of tumor are used.

The next advance of interest was the determination that this condition of more or less perfect immunity may also be produced by the injection of certain normal tissues from animals of the same species. Bashford, Murray and Cramer found that mice previously treated with the whole blood (or simply with the red blood cells without serum) of normal mice were protected from mouse tumors in a large percentage of cases. These results have been confirmed by Bridré for mouse tumors and by Lewin for rat tumors. Schone[16] obtained similar protection by injecting mouse embryos. Bridré found an almost perfect protection following the injection of mouse spleen and a somewhat less one following mouse liver. The pan-immunity of Ehrlich which followed the implanting of virulent hemorrhagic tumors and the failure of these tumors to take in many instances may be attributed, according to Gierke, to an immunity caused by the blood in the tumor.

The exact nature of this immunity following resorption of a cancer or implantation with cancer or normal tissue has been attentively considered. Bridré has described it as an "iso-immunity to mouse tissue." The original assertion of Gaylord, Clowes and Baeslack[17] that the serum of recovered mice will cure cancer in other mice, or, when mixed with cancer, prevent its growth, has received no confirmation. The experiments of Crile and Beebe[18] on transfusion of blood from dogs recovered from lymphosarcoma into dogs suffering from the same tumor, followed in certain instances by an apparent cure, do not seem to me to indicate necessarily the presence of immune principles in the blood of the donors. Controls transfused with blood from normal dogs seemed to give almost equally good results and the fact that in most of the cured cases more blood was transfused than had been removed from the recipient would seem to be a factor of even more importance than they have given it. There are, moreover, several observations which would seem to prove that the blood of such refractory animals does not contain actual antibodies for cancer cells. Michaelis[19] studied the serum of actively immunized mice and found that it did not agglutinate nor destroy cancer cells. He found, moreover, that a combination of this serum with cancer extract effected no alexin (complement) fixation. Sticker found that the blood of dogs recovered from his sarcoma did not agglutinate sarcoma cells. Flexner and Jobling found that the serum of recovered or repeatedly implanted rats had no power to protect normal rats from their tumor.

It is naturally a matter of extreme importance to determine whether this acquired or intensified immunity is similar to the usual form of immunity against bacteria, that is, whether it is characterized by the presence in the serum of immune substances antagonistic to cancer cells. The most delicate means of detecting such antibodies is the Bordet-Gengou "reaction of fixation" method. The reaction depends essentially on the fact that the complex, immune body plus antigen has the power to absorb the alexin (complement) of fresh serum, the absorption or non-absorption being proved by the absence or presence of hemolysis in subsequently added, sensitized red blood cells. In the instance in question the fixation of the alexin in fresh serum by a combination of cancer extract and the serum of a refractory mouse or rat would prove the presence of antibodies to cancer in that serum. I have performed a number of experiments of this sort fully controlled in the manner indicated by the following experiment:

EXPERIMENT I.

A refractory Rat F. E. 12 (having received two implantations of the tumor without result, the last time sixteen days previously) and a normal rat were bled from the carotid on May 18. The respective sera were separated from their clots on the following day and heated to 56° C. for one-half hour (to destroy alexin). .1 gram of rat cancer that had been dried at 37° C. was shaken up in three cubic centimeters of NaCl solution (.85 per cent) and after repeated filtration used as indicated in preparing the following tubes:

Tube	1.	Serum of refractory rat, 56°,	.3 cc.*	+ cancer extract	.05 cc.†
“	2.	“ “ “ “ “	.2 “	+ “ “	.05 “
“	3.	“ “ “ “ “	.1 “	+ “ “	.05 “
“	4.	“ “ “ “ “	.3 “	+ NaCl sol.	.5 “
“	5.	“ “ “ “ “	.2 “	+ “ “	.5 “
“	6.	“ “ “ “ “	.1 “	+ “ “	.5 “
“	7.	“ “ normal “ “	.3 “	+ cancer extract	.05 “
“	8.	“ “ “ “ “	.2 “	+ “ “	.05 “
“	9.	“ “ “ “ “	.1 “	+ “ “	.05 “
“	10.	“ “ “ “ “	.3 “	+ NaCl sol.	.5 “
“	11.	“ “ “ “ “	.2 “	+ “ “	.5 “
“	12.	“ “ “ “ “	.1 “	+ “ “	.5 “
“	13.	NaCl.	1 “	+ cancer extract	.05 “

To each tube was then added .1 of a cubic centimeter of fresh rabbit serum (eighteen hours old) and after contact for five hours at room temperature, .5 of a cubic centimeter of a five per cent suspension of sensitized guinea-pig corpuscles ‡ was added to each tube. Hemolysis (two hours at 37° C.) was complete in all tubes but 1, 2, and 7 in which latter it was shortly after complete.

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*Tubes in this column are made with salt solution to equal in volume one cubic centimeter.

†Tubes in this column are made to equal in volume .5 of a cubic centimeter.

‡This is washed guinea-pig's blood in five per cent suspension that had been mixed for several hours with the inactivated (56°) serum of a rabbit that had received four injections of guinea-pig blood (hemolytic serum). Proportions fifteen cubic centimeters blood suspension to three cubic centimeters of serum.

There is then no distinct evidence that the blood of this refractory rat contained any antibodies for cancer cells.

In some experiments the extract obtained by maceration from a fresh tumor was also tried in place of the extract from dried tumor. The factors involved (antigen (cancer extract) and the suspected antiserum) were varied in amount; another reactivating serum (alexin) was tried; and other hemolytic combinations employed (viz., rabbit blood plus antiserum from the guinea-pig, and sheep blood with antiserum from the rabbit).

In this manner the blood serum of six different rats that had been rendered highly refractory to the tumor and also of three animals of a normally refractory strain were tested for antibodies and none were found. The "pooled" serum of several mice that were rendered refractory to the Ehrlich tumor (Stamm II.) was also tested with this tumor with a negative result.

There is no evidence, then, that the natural or acquired refractoriness of certain rats to the tumor is due to the presence of such antibodies as are responsible for the ordinary anti-bacterial immunities. It seemed possible, however, that the blood of such refractory animals might still have some preventive or inhibiting effect on tumor growth, and a number of experiments have been undertaken to determine this point. Some of these experiments were done on the supposition that all races of rats would prove equally susceptible to the tumor under normal conditions and therefore do not answer the question as put. But the experiments on rats that were later, from the controls, found to be insusceptible, are of distinct interest, as will be evident. All of the animals in the following series were of similar weight (50–75 grams).

EXPERIMENT II.

The effect of the previous injection of blood in insusceptible animals followed by cancer. Nine rats from dealer "H" were treated as follows:

Lot 1. 3 rats. No treatment (Controls).
Lot 2. 3 rats. Given each 1.5 cubic centimeters defibrinated blood of normally susceptible rats.
Lot 3. 3 rats. Given each 1.5 cubic centimeters defibrinated blood of refractory rats.*

Nine days later all were implanted with pieces from the same tumor with the following result:

Lot 1. No tumors grew.
Lot 2. 1 tumor grew.
Lot 3. 2 tumors grew.

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* That is, rats which had failed to grow the tumor and had been reimplanted once or twice with the tumor.

It is evident from the controls (Lot 1) that these rats are normally insusceptible to the tumor. The previous injection of blood of susceptible rats and particularly of refractory rats would seem to facilitate growth of the tumor.

Experiment III.

The effect of the simultaneous inoculation of various bloods and cancer in insusceptible animals.

Twelve rats, source "P," were given each 1.5 cubic centimeters of the blood indicated on the same day on which they received an implantation of cancer. The blood was injected on the side opposite to the cancer implantation.

Lot 1. 3 rats. Cancer alone (Control). 1 tumor.
Lot 2. 3 rats. Cancer + blood normally susceptible rats (C). No tumors.
Lot 3. 3 rats. Cancer + blood normally insusceptible rats. 2 tumors.
Lot 4. 3 rats. Cancer + blood refractory rats. 3 tumors.

It would certainly seem indicated from these experiments particularly the second, that the blood of insusceptible or of refractory animals injected previously, or at the same time as the implantation of the cancer in normally insusceptible animals, would lead to an increase in the number of takes. This is controlled both by the non-injection of blood and the injection of normal susceptible blood.

The results with relatively susceptible rats "L" and similar bloods are quite different.

Experiment IV.

Simultaneous injection of blood and cancer in moderately susceptible rats.

a. 4 rats given cancer alone. 2 tumors.
4 rats given cancer + susceptible blood. 4 tumors.
4 rats given cancer + refractory blood. 1 tumor.
b. 5 rats given cancer alone. 3 tumors.
5 rats given cancer + susceptible blood. 2 tumors.
5 rats given cancer + refractory blood. 4 tumors.

Obviously no conclusions can be drawn from these latter experiments. The one point of interest seems to be the apparent stimulating effect to tumor growth in insusceptible animals by the injection of the blood of refractory animals.

These facts all go to show that the immunity or refractory condition in animals to cancer is not explicable along the lines of ordinary anti-bacterial immunity.

It is evident that this resistance is dependable on very slight variations when we consider that mere change of locality may cause an insusceptible animal to become susceptible (Haaland). This led me to consider a factor that has seemed to be of some importance in another form of "iso-immunity." I[20] found that the isoagglutination of human blood was accompanied by certain differences in the tonicity of the bloods in question. For instance, if the blood serum of "A" agglutinates the red blood cells of "B" it will be found that the corpuscles of A and B differ in the extent to which they undergo dissolution in salt solutions of various concentrations. A careful comparison of the defibrinated blood of several normal, refractory, and cancer-bearing rats failed to show any consistent variations in their resistance to hypotonic solutions of sodium chloride.

Ehrlich has offered an explanation of cancer immunity which for a time at least seemed rather plausible. Although the successful transplantation of the tumor of one species in an animal of another species is usually impossible, Ehrlich found that when a mouse tumor was transplanted in a rat it would grow for a period of, say, eight days and then begin to retrograde. This mouse tumor that has grown in the rat "takes" perfectly well when brought back into the mouse and may subsequently be grown for a similarly brief period in another rat. And again Ehrlich states, on the basis of numerous experiments, that a mouse bearing one tumor will not grow a second implanted tumor. Largely on the basis of these two sets of observations he has offered his theory of "atreptic immunity (*α* privative and *τρέφω* = I nourish). According to this hypothesis the growth of a tumor depends on the presence of certain "X" substances, which directly or indirectly furnish a specific nourishment for cancer cells. The mouse tumor will grow in a rat as long as the "X" substance brought over with the tumor lasts; these substances are replenished on second growth in a mouse and the tumor may then grow in another rat for a time. Similarly the failure of a secondary implanted tumor to grow in Ehrlich's cases would be referable to a monopoly of the "X" substances by the original tumor.

A consideration of this atreptic theory has led to numerous results of interest. In the first place Ehrlich's general assertion that a second implanted tumor will never grow has been entirely disproved. Bridré found that second mouse tumors grew perfectly well, and both Lewin and Flexner and Jobling get a growth of second tumors in rats. Loeb regards a tumor-bearing animal as even more susceptible to tumors than a normal one, with which statement Gierke agrees. Simultaneously inoculated tumors both grow equally well (Bridré, Gierke, and myself). Schone[21] found that if he removed a growing tumor and a week or two later implanted a second one it invariably grew. Sticker divided the phases of tumor growth into two distinct periods, in the first, during which there are no metastases, a second implantation grows only when the first tumor is removed. During the second period (metastatic) a second tumor will grow as well as in a normal animal. Similarly, Flexner and Jobling found that their second implant would grow in a larger number of cases during this second period.

I had, independently of Schone's observation, already made some experiments as to the effect of tumor removal on the growth of a second tumor. In my own experiments I have implanted a second tumor on the opposite side immediately after removal of the primary tumor. As the age of growth of the first tumor is of some importance it has been included:

TABLE II.

On the growth of a second tumor implanted at once after removal of a primary tumor.

Rat No.	Age of Tumor Removed.	Second Tumor.
1....................	16 days.	No growth.
2....................	28 "	" "
3....................	28 "	" "
4....................	30 "	" "
5....................	30 "	" "
6....................	30 "	" "
7....................	30 "	Growth.
8....................	30 "	Growth.

It would seem, then, as if the second implanted tumor fails to grow after removal of the first tumor in the majority of instances up to thirty days. The probable relation of the presence or absence of metastases to these results can be determined only on fuller evidence as to growth of the second tumor after longer periods of growth of the first.

Of far greater interest is the result of experiments which were designed in the first place to control these results of reimplantation after removal. In order to control the effect of removal as compared with non-removal of the original tumor, animals were left with the original tumor and a second implanted. In a few of these animals the second tumor grew, but in most it did not, and in these latter animals it was found not only that the second tumor failed to grow but that in many instances the original tumor disappeared.

EXPERIMENT V.

Four rats in a given experiment showed tumors that had been implanted twenty-eight days previously on the left side. The largest of these tumors measured one centimeter in diameter. There were also on hand four normal animals of the same strain that had never been inoculated.

All eight animals were implanted with tumor F. F. 1 on the right side with the following result:

4 normal rats.	Tumors grew in 3.
4 tumor rats.	Second tumor grew and original continued to grow in 2. Second tumor did not grow and original tumor disappeared in 2.

The controls show that the second tumor was viable, inasmuch as three of the four animals gave positive growths. The growth of the second tumor in two of the tumor rats further evidences this fact.

It is unfortunate, as will later appear, that no histological material was saved from these two animals. The point of greatest interest, however, is the fact that in two tumor animals in which the second tumor failed to grow the first tumor actually disappeared.

A more striking instance of the disappearance of the original tumor apparently due to the implantation of a second tumor is shown in

Experiment VI.

Ten rats of a given lot that had been implanted with tumor thirty days previously showed tumors of from one to two centimeters in size, and were reimplanted with tumor on the other side of the belly. The original tumor was removed in five of these animals just before the second implant; as already noted (Table II.) in two of these animals the second tumor grew. In the other five the original tumor was left and the second implantation gave the following result:

In one animal the second tumor grew and the first tumor continued to grow.
In four animals the second tumor failed to grow and the first tumor disappeared.

These experiments indicate very strongly that the implanting of a second tumor may result in many instances in disappearance of the original tumor as well as in a failure of the second to grow. As this result was unexpected the experiments were none of them carried out in such a way as to make this "cure through vaccination" appear to the best advantage. Certain controls were omitted. It may be questioned, for example, as to whether or not the original tumors might not have disappeared spontaneously. The data at hand in the form of histories of individual animals make it seem most probable that tumors marked as "positive" on the fourth week rarely disappear; and yet experiments to show "cure" should unquestionably include such control animals. In later experiments a careful histological control of the animals in which the original tumor entirely failed to disappear renders it very evident moreover that even in such animals a degree of "cure" had been effected by reimplanting the tumor.

EXPERIMENT VII.

On July 21st forty rats from dealer "L," weighing on an average fifty grams each, were given implantations of tumor F. F. 51 on the left side. On the twenty-first day there were thirteen of these animals with definite tumors that were then divided into two lots:

Lot 1. Five animals with the *smallest* tumors.
Lot 2. Eight animals with larger tumors.

The animals of Lot 1 received no further treatment. The animals of Lot 2 received reinoculation of tumor on the right side on the twenty-first day and on the back on the twenty-fourth day.

The resulting growths were as follows:

Lot 1.
1. Died 80th day and was partially eaten.
2. Died 90th day. No examination possible; eaten.
3. Killed 120 days. Tumor measures 2.5 x 2 centimeters and is firm with living periphery. No metastases.
4. Killed 120 days. Tumor measures 2 x 2 centimeters and is firm and living.
5. Killed 140 days. Tumor 1.5 x 1 centimeters, firm. Histologically it resembles the usual good growing tumor with numerous mitoses. (See Fig. 1.)

Lot 2. Nos. 1, 2, and 3 died shortly after reinoculation and were eaten so that no examination was possible.

Nos. 4, 5, and 6. By the sixtieth day the original tumor had disappeared and did not recur subsequently. The reimplanted tumors failed to grow.

7. Died on 65th day. No apparent cause. The original tumor measures 1.5 centimeters in diameter and is yellowish white in color. Histologically the nodule is composed of fibrous tissue containing a few strands of epithelial cells about the periphery. (See Fig. 3.)
8. Killed on 95th day. All three tumors are present and do not differ from the ordinary type except in being more necrotic than usual.

Allowing for those animals in which examination was impossible through accident the experiment may be summarized:

In all three controls the tumor grew.

Of five treated animals the tumor disappeared entirely in three and was nearly replaced by connective tissue in a fourth when it died; in none of these four did the second or third tumor grow. In the fifth animal both the second and third tumors grew and the first failed to disappear.

EXPERIMENT VIII.

Sixteeen "L" rats were given tumor F. F. 28 on July 9th. On the thirty-third day nine of these animals showed definite tumors and were divided into lots and treated as follows:

Lot 1. Three animals with the smallest tumors (largest 1.6 x .7 centimeters and smallest 1.3 x .8) were given no further treatment.

1. Died 37th day. No examination possible.
2. Killed 90th day. Large tumor with growing periphery and necrotic center. No metastases.
3. Tumor failed to grow.

Lot 2. Six animals with largest tumors (varying in size from 2.8 x 1 centimeters to 1 x 4 centimeters) were given tumor F. F. 46 on right side and three days later tumor R. 2 on back.

Nos. 1 and 2. On the 90th day and subsequently no tumors were present.

3. Killed 90th day. There are the remains of the first and second tumors which are necrotic. Histologically the first tumor shows granulation tissue but no epithelial cells. In the second tumor there are the remnants of a few necrotic epithelial cells.
4. Killed 90th day. Two necrotic areas mark first and second tumors. The few remaining tumor cells are necrotic and vacuolated with pyknotic nuclei. There is no increase of connective tissue. (See Fig. 2.)
5. Killed 90th day. Third tumor alone evident. Histologically it shows degenerated epithelial cells and granulation tissue.
6. Killed 90th day. Remains of two tumors present. In the first there is increased connective tissue about the periphery and thin strands of epithelial cells. The other tumor is similar.

In this experiment the tumor failed to grow in one of the two controls which survived.

In two of six treated animals no tumors were left on the ninetieth day. In the remaining four, killed on the ninetieth day, the epithelial cells were degenerating and had all but disappeared in three, and were largely replaced by connective tissue in one.

The cumulative evidence from these four experiments, each of which is fairly conclusive in itself, would seem to show very definitely that the reimplanting of a tumor in a tumor-bearing animal twenty-one to thirty-three days after the first tumor will usually lead to a disappearance of the original tumor and a failure of the implanted tumor or tumors to grow. Thus, in twenty treated animals the tumor disappeared in twelve, had entirely degenerated or been replaced by connective tissue in four, and failed to be affected by the reimplantation in four cases only; in these latter cases it is to be noted that the reimplanted tumors also grew.

It is only fair to describe at once the only other experiments of this nature that have been performed and which failed to give as marked evidence of cure after reimplantation for reasons that are not quite evident.

Experiment IX.

Thirty-four "L" rats were given tumors on left side, and forty-one days later sixteen showed positive tumors. Six animals showing smallest tumors were left untreated. The tumor continued to grow in all. They were killed at intervals from the one hundred and ninth to the one hundred and fifty-sixth day, and four of the six showed metastases. The ten remaining tumor rats were given tumors on the forty-first and on the fifty-second day. When killed they showed the following conditions:

1. Killed 110th day. 3 tumors. Metastases.
2. Died 112th day. 1 tumor. No metastases.
3. Killed 112th day. 2 tumors. Metastases.
4. Killed 112th day. 2 tumors. No metastases.
5. Killed 112th day. 2 tumors. No metastases.
6. Killed 141st day. 2 tumors. No metastases.
7. Killed 143d day. 2 tumors. No metastases.
8. Killed 143d day. 2 tumors. No metastases.
9. Killed 143d day. 2 tumors. No metastases.
10. Killed 143d day. Small original tumor. No metastases.

The histology of the tumors from 1 to 6 inclusive resembled that of the controls. The tumors on the last four animals, however, showed a distinct increase in connective tissue over the tumors in the control rats. It is also of distinct importance to note that metastases were present in only two of the ten treated animals whereas they were shown by four or six controls. The late period at which the treatment was begun in this experiment (forty-one days) is also a factor of probable importance. Whereas this experiment cannot be used as a definite proof of the curative value of reimplantation it is certainly indicative of an attempt at cure.

Experiment X.

Fifteen young "L" rats, average weight seventy grams, were given tumor from F. G. 28 on left side October 26. Of these, six showed distinct tumors of approximately the same size on the twenty-fourth day and were divided into two equal lots and treated as follows:

Lot 1. Controls. No further treatment.
Lot 2. Given tumor from F. G. 29 on right side.

They were all chloroformed on the fifty-eighth day and showed the following picture:

Lot 1:
1. Firm living tumor. Metastases in lungs.
2. Tumor 2 x 2.5. No metastases.
3. Tumor 3 x 2.5. Metastases in lungs.

Lot 2:
1. Died before this date. No examination.
2. No tumors. Pin head connective tissue nodule indicates original tumor. No metastases.
3. Original tumor shows considerable amount of living tissue. No second tumor. No metastases in lungs. Lymph node shows in growth of tumor.

Three controls were positive. One animal of two treated was cured, the other showed no distinct evidence of any reaction to implantation.

Experiment XI.

On October 24th sixteen young "L" rats, weighing on an average seventy grams, were given tumor from F. G. 94 on the left side. Twenty-four days later seven of these animals showed positive tumors and were divided into two lots as follows:

Lot 1. 3 rats with tumors averaging 1.5 x 1.2 centimeters (largest 1.8 x 1.6, smallest 1.2 x 1) were left untreated as controls.

Lot 2. 4 rats with tumors averaging 2.2 x 1.3 centimeters (largest 3 x 1.5 centimeters, smallest 1.9 x 9) were given tumor F. G. 29 on right side.

One treated animal died in a few days. The remaining rats were chloroformed on the sixtieth day with the following findings:

Lot 1:

1. Firm growing tumor. Metastases in lungs.
2. Small firm tumor. No metastases.
3. Large necrosed tumor. No metastases.

Lot 2:

1. First tumor necrotic; second 1.5 x 1 centimeters. No metastases.
2. First tumor 3 x 2.5; second 3 x 1.5 centimeters. Metastases in lungs.
3. First tumor 3.5 x 2.5; second 2.5 x 1.5 centimeters. Metastases in lungs.

Histologically there were no differences between the tumors of treated and untreated animals. There is then no evidence of cure in this experiment.

Reinoculation of tumors in tumor-bearing mice has, of course, been tried repeatedly since Ehrlich's statement that a second tumor would not grow. Those writers who have considered this question have, however, failed to more than touch on the possibility of a cure of the first tumor through implantation. That there may be a stage (pre-metastatic period of Sticker) in the growth of a tumor during which reimplantation will lead to resorption of the original as a rule, has apparently escaped their notice. Bridré, to be sure, notes in a few sentences an apparent cure which followed injections of fresh tumor maceration in one or two animals, and Gierke[26] mentions in a footnote an unpublished communication of Bashford as to the possibility of rendering tumor animals immune by treatment with badly growing spontaneous tumors. The general experience has been, however, that a tumor would take better in a tumor-animal than in a normal one. Such a statement doubtless depends on experiments performed during the metastatic period.

The suggestion has already been made as to the possible effect of a metastatic period on this "cure by vaccination." According to Sticker during the first "non-metastatic period" no second implant will take, and it would also appear from my results that during this period this second implant may lead to cure. We may regard this first period as present as a period of adequate defence to invasion on the part of the host. It is of great interest to note that in human beings suffering from cancer, and often they were very early cases, that a specific "reaction of fixation" between the patient's blood serum and cancer emulsion has been found both by Ludke,[22] and more recently by Simon and Thomas.[23] As already mentioned, both Michaelis and I have found no such reaction with the serum of refractory rats. A summary of some experiments, performed and controlled as already detailed, with the serum of cancer-bearing rats with note as to the presence or absence of metastases is of interest.

TABLE III.

Reaction of fixation experiments with the serum of cancer rats.

Rat No.	Age of Cancer.	Metastases.	Result.
F. E. 4	16 days.	0	0
F. G. 88		0	0
F. E. 1	34 days.	0	+
F. G. 89		0	0
F. E. 17	34 days.	0	0
F. G. 28		0	+
F. E. 9	37 days.	+ +	0
F. E. 2	37 "	+	0
F. D. 15	37 "	+ +	0
F. G. 92	156 "	+	0

Thus in two cancer animals that had no metastases a reaction of fixation was present. No such reaction was obtained during the metastatic period. The presence of an active resistance on the part of the tumor animal during the premetastatic period as indicated by a reaction of fixation, and an absence of such resistance during the metastatic period, would explain the reason for a cure when the animals are "vaccinated" with a new tumor during the first few weeks as well as why the second tumor grows during a later metastatic period.

SUMMARY AND CONCLUSIONS.

In the majority of my experiments in transmissible tumors I have employed the Flexner-Jobling tumor of the rat. As it occurred originally this tumor was described as a sarcoma, but later was mentioned by the authors in question as having changed to a carcinoma, which was its character when it came into my hands. It has showed no marked histological changes during the eight generations through which I have cultivated it.

The susceptibility of white rats obtained from various sources to this tumor varies remarkably. Whereas the animals from one source gave one hundred per cent of "takes" the next most susceptible strain gave only fifty per cent of positive results.

Following successful subcutaneous implantation in the region of the axilla, metastases occur regularly in the lungs and only very rarely in the adjacent lymph nodes. The rapidity with which metastases occur would seem to depend on the susceptibility of the strain of rats employed and in the most susceptible strain would seem to occur at a rather definite period after implantation.

Successive transplantations from lung metastases in the most susceptible strain apparently lead to an increased virulence in the tumor, as indicated by a rapidity in occurrence and increased number of metastases, as well as by a distinct increase of the epithelial constituents of the tumor over the stroma.

Animals that have failed to take the tumor on first inoculation are very seldom susceptible to a second or third implantation. The blood serum of animals rendered refractory by several implantations, as well as the serum of animals of a normally insusceptible strain, gives no fixation of alexin with an extract of the tumor employed. No distinct preventive effect is evident on simultaneous injection of such "refractory" blood and cancer in animals that are naturally susceptible. This refractory blood when injected previous to or at the same time as implantation of cancer in normally insusceptible rats would seem definitely to increase the percentage of takes.

The bloods of normal, of cancer-bearing, and of refractory rats show no difference in tonicity as measured by the resistance of their respective blood corpuscles to hypotonic solutions of sodium chloride.

Following the removal of a tumor that has been growing for from sixteen to thirty days a second implantation of tumor usually fails to give a positive result. A few animals on the thirtieth day gave a positive second tumor. This period extending to the thirtieth day corresponds rather closely to the limit of the period during which no metastases are found (Sticker).

When the primary growing tumor is left and a second tumor implanted during the "pre-metastatic" period, in many instances the second tumor not only fails to grow but the first tumor entirely disappears. In cases in which resorption of the original tumor was incomplete when the animal was killed, either from lack of sufficient time for the effecting of a complete cure or in a case of incomplete cure (in which case the second tumor may grow), the original tumor shows distinct histological changes. These changes are evident either in the form of degeneration and vacuolization of the protoplasm of the cancer cells with pyknotic changes in their nuclei, or else by the occurrence of an enormous increase of connective tissue which compresses the remaining strands of epithelial cells. No such changes have been found in non-treated tumors.

A reaction of fixation has been found between cancer extract and the blood serum of a few cancer-bearing rats during the pre-metastatic period, but never during the metastatic period. The pre-metastatic period, then, would seem to be characterized by an active defence on the part of the animal as indicated by the presence of antibodies. During this reactive period, reimplantation will increase the resistance to such an extent that the original tumor is destroyed. When this period is passed metastases occur and a second implanted tumor grows.

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DESCRIPTION OF PLATE IV.

FIG. 1. — Ordinary tumor growing one hundred and forty days in a non-treated rat (compare with Fig. 3). Normal relations of cancer cells and stroma.

FIG. 2. — Original tumor from rat that had received reimplantations on thirty-third and thirty-sixth day. Killed on ninetieth day. Epithelial cells show degenerated and vacuolated cytoplasm and pyknotic nuclei.

FIG. 3. — Transplant from same tumor as rat in Fig. 1 in another rat of same strain that was treated with a second and third implantation. Sixty-five days after implantation of tumor (compare with Fig. 1). Marked increase of connective tissue stroma with compressing of columns of epithelial cells.

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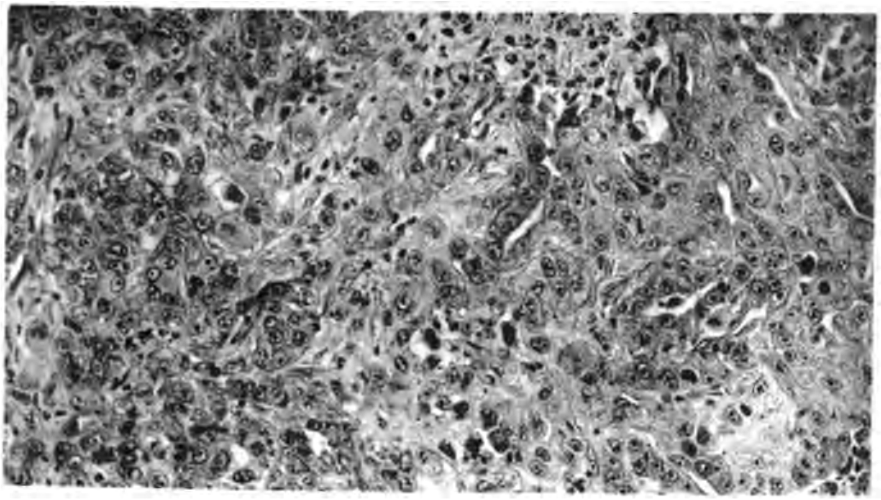

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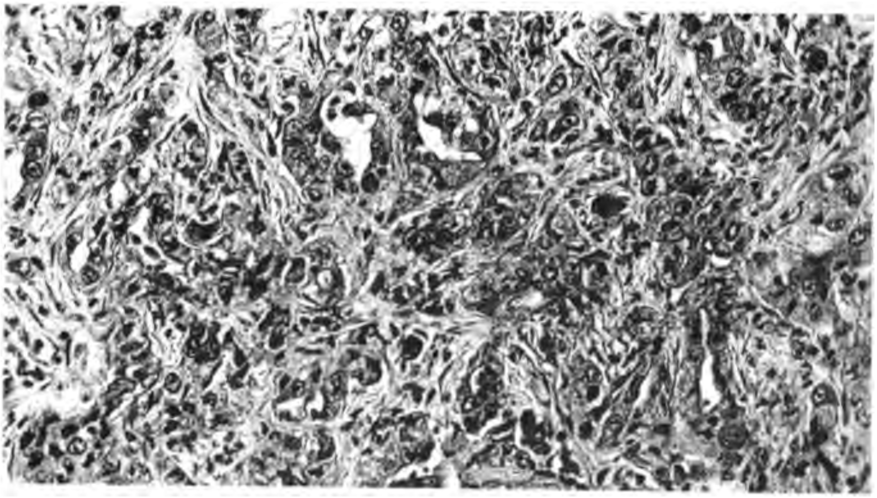

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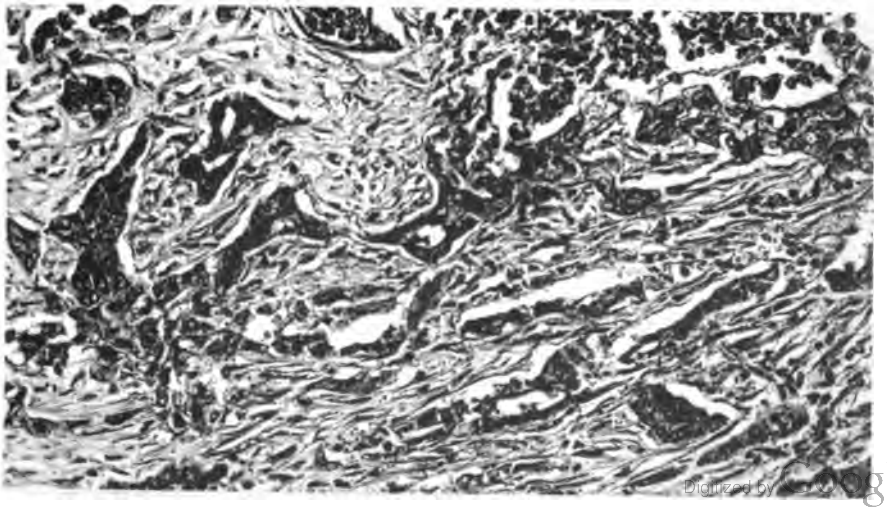

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Gay. Cancer of rat.